nature 29 June 1978

Hiring and firing gets more complex

TWENTY, even ten years ago the personnel management side of industry or indeed of any employer was a relatively minor affair. Employers hired when they needed new people and fired when the need had gone away or if they felt their employees were incompetent. The system was undoubtedly harsh, particularly on manual workers who could be dumped almost at a moment's notice, and few would deny that the recent trends in many countries towards more job security have been humane and have reduced drastically the opportunities for exploitation. But few would also deny that these trends, enshrined in employment legislation, have not only placed far greater demands on personnel management but have also tied employers' hands to a degree that can restrict ability to survive in a rapidly changing world.

It is certain that when legislation is being framed the last people who are likely to be in parliaments' minds are university research staff, yet it is amongst this community that some of the least desirable backwash from the legislation can be found. There are two reasons for this—that science moves quickly and may cruelly strand its practitioners in a world they cannot cope with, and that modest ability, even coupled with a strong drive, is often insufficient in a small group in which every member must play a major role. You can stop employers exploiting by means of legislation, but science itself, by its rapid and unpredictable methods of evolution, in a sense exploits its own practitioners in a way that cannot be legislated against. So what happens when research institutions, the presumed guardians of the spirit of science come up against employment legislation? In recent months a clear conflict arose in the United States when a nationwide raising of the age to which people could work if they wished produced cries of horror from universities aghast at the thought of five more years' employment from professors whose involvement with scholarship had waned, to say the least.

In the UK, where the elderly form nothing like as strong a lobby, the pressure, if anything, is to lower the retirement age, but that does not mean that there are not other employment pitfalls. Take, for instance, the hiring of postdoctoral workers, research assistants and technical staff on short-term contracts. For many years this has been a well-tried procedure for giving younger scientists their first taste of self-propelled research as a testing ground for suitability for more permanent employment. In recent years with a decline in the number of permanent posts coming on to the market and with a reduced willingness amongst scientists to move around, short-term posts on research grants have taken on a greater significance and have often had to be the holding pattern in which excellent young scientists have to circle for years and years before they can alight on a permanent position. But with recently enacted legislation, particularly the Redundancy Payments Act and the Employment Protection Act, things are not so easy as they used to be.

Case history on fixed-term contracts is still being established in the courts, but the pattern that seems to be emerging is that a contract between employer and employee for a fixed period is to be regarded as just that—neither side is at liberty to terminate the contract by giving notice except in the case of a fundamental breach such as wilful misconduct by the employee. In theory an employee who has done, say, two and a half years of a three year contract and then is offered a post elsewhere is unable to take up this new post until the contract has expired, although in practice blind eyes are being turned.

Nor is it entirely simple to end the contract even when its nominal period has expired. These days expiry of a fixed-term contract is regarded as dismissal, and it is open to an employee to attempt to claim for unfair dismissal or to seek redundancy payments. Employers are, however, at liberty to ask potential employees to sign a clause in their contracts waiving their rights to such claims.

Those administrators who are trying to establish satisfactory new contracts describe the scene as a legal minefield. Scientists who are in the position of employing people on short-term contracts are divided as to how much the new legislation affects research. Opinions vary from the expression of the view that it could 'make it very difficult to undertake research on three-year grants' through to the assertion that it will simply 'make sure that we take care to hire the right people in the first place'. There is little doubt, however, that another small restraint has been imposed on freedom to do research. □

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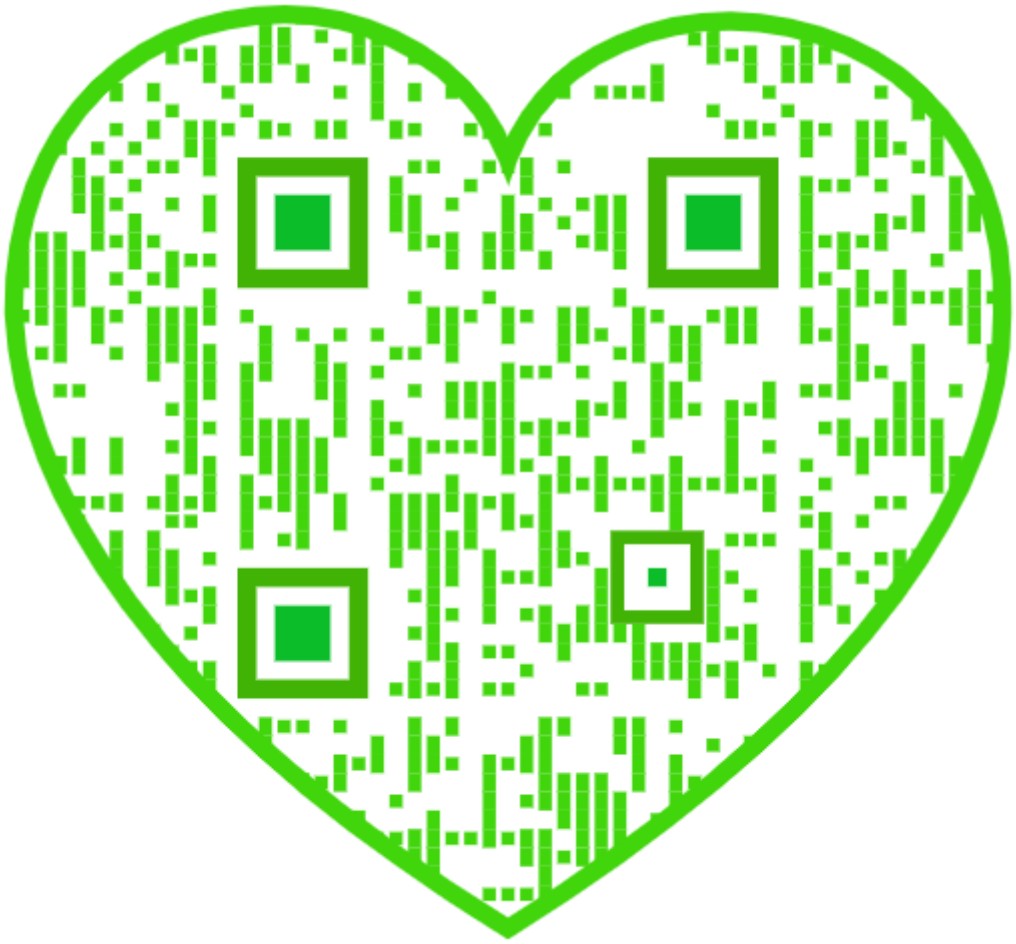

Recombinant DNA is safe

Professor R. H. Pritchard argues that there are no real hazards in recombinant DNA research.

THE publication of the first report by the Genetic Manipulation Advisory Group has been the occasion for a number of articles in the press giving muted praise for the unprovocative, unemotive and constructive approach of we British to the problems posed by the attempt to control research with recombinant DNA. The Group has had an unenviable task and I would not wish to minimise the difficulties it has had in attempting to formulate rules that are acceptable to everyone concerned. Nevertheless, the occasion must not be allowed to pass without comment on the less commendable features of its Report and on the present level of debate on this issue in Great Britain.

The only conceivable justification for the expensive and growing bureaucracy which now controls recombinant DNA research would be that it posed an identifiable and distinct hazard to human health or society. Consequently it cannot be emphasised too often that no such hazard has ever been identified. Nor have I heard a sustainable theoretical argument to suggest that such hazards are real. Recent comment in the press and Parliament indicates a total misunderstanding of this point as witnessed by the fact that the so-called hazards have almost invariably been compared with those surrounding the nuclear energy industry. The difference between the two is unambiguous. The hazards of radiation are known from experience of them. They are quantifiable, and appropriate control can therefore be based on reasoned argument.

GMAG and other official bodies that have preceded it must take substantial responsibility for allowing such a widespread and fundamental confusion to exist and to continue to exist. Indeed, it is most disturbing that the GMAG report, if only by default, leaves the impression that there is broad agreement about the basic premises upon which its existence is based and that it need only concern itself with the definition and execution of regulations based upon these premises. Discussions I have had with many biologists indicate that some regard the recombinant DNA debate as the longest running and most expensive farce in town. Others accept the need for caution and control in the execution of certain kinds of experiments but believe that the GMAG guidelines are far too sweeping. Others believe that by confining their attention to *in vitro* recombinant DNA experiments the Group is operating in a logical vacuum with the result that if some of the conjectured hazards are real then the horse will probably have bolted before ever it enters their stable door. I have yet to meet a biologist who approves of the proposals as they stand. None of this divergence of views would be apparent to those readers of the GMAG Report who are not biologists, although it is they who will be most influenced by it.

The total absence of reasoned argument based on observations has led to the most disturbing aspect of the whole debate. This is the continual resort to authority rather than to evidence as a justification for control. GMAG summarising the background to its birth tells us "In July 1974 a group of distinguished biologists drew public attention to these problems" (the hypothetical risks of recombinant DNA research). And later (para. 4), "The question of hazards was raised by responsible scientists and we feel it essential that, however strong an individual worker's intuitive feeling may be that the hazards have been grossly overestimated, we have a right to expect of him, until we know more of the reality of the situation, that he should do the work either with scrupulous care or not at all". Mere workers, apparently, who disagree with "distinguished" and "responsible" scientists can only have "intuitive" reasons for doing so.

I do not wish to question the distinction of the scientists concerned, but clearly it is they who acted upon intuition. It is my personal opinion that their intuition was unsound, that they were ill-informed and consequently, in view of their distinction, it is they who acted irresponsibly. The soundness of the suggestions made by these scientists has never been rigorously discussed by those in this country who have been asked to do so. The Working Party chaired by Lord Ashby was unable to identify the hazards of recombinant DNA research. The following Working Party under Sir Robert Williams nevertheless felt able to divide experiments into four categories of increasing risk. Now we find GMAG debating in its report whether DNA from birds should be categorised in terms of its hazard level with mammalian DNA (because birds are warm blooded) or with amphibian DNA (because of the closer evolutionary relationship). One is tempted to wonder why the group didn't consult the I Ching for advice.

Practising biologists will not find it easy to adhere to restrictive and time-consuming rules, and ensure that others do too, when there is so little confidence that these rules have a rational basis. Consequently, the question that now concerns us most is on what evidence and by what means will the controls be relaxed. On this question as on others surrounding the recombinant DNA debate there seems little ground for optimism.

Consider, for example, the suggestion by the distinguished scientists that the creation of novel antibiotic resistant phenotypes among bacterial species by *in vitro* recombination is a potential hazard. Antibiotic therapy has been in widespread use for decades. A consequence has been the development of resistant strains of pathogens and non-pathogens. Such strains can become the prevalent ones in hospital environments and are a hazardous nuisance. I am not aware, however, of a single example in all this time of *any* bacterial species in which resistance to *any* antibiotic has become the prevalent phenotype except in an antibiotic-contaminated environment. Is there a reasonable alternative to the supposition from these observations that there is a price to be paid for antibiotic resistance by a naturally sensitive species and that this price is too high for resistant strains to predominate without positive selection in their favour? If resistance confers no selective advantage, what is the cause for concern except the one which has always been with us? This is how best to employ antibiotics so as to take maximum advantage of the natural selection pressures *against* resistant organisms to minimise their prevalence. Had the resources devoted to the recombinant DNA debate been devoted instead to investigation of this latter question, an important medical problem might have been solved. Instead a bureaucracy has been created.

But to return to relaxation of controls. Is it likely that GMAG, or any other body, will ever have in its possession more convincing evidence than it has now that *in vitro* transfers of antibiotic resistance determinants present no hazards? I suspect not and therefore wonder how relaxation of the blanket controls of this kind of activity are ever to be relaxed if they are not to be removed immediately. Moreover, if relaxation here proves a problem, then how much greater a problem will it prove to be in the case of the more esoteric areas of conjecture?

Great Britain will, from 1 August, have the doubtful privilege of being the first country to bring recombinant DNA research under legislative control. I find it hard to understand why GMAG has not publicly and firmly advised that this legislation be deferred until there is evidence, or even broad agreement, that the postulated hazards do indeed exist. □

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Commonwealth Science Council coordinates approach to UNCSTD

Alastair Hay assesses the CSC effort to produce a commonwealth view on development

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Hubertus Kanus

Rope making in Pakistan: as technology becomes more complex, dependence on the first world experts grows

WITH nearly 150 countries preparing papers for the forthcoming United Nations Conference on Science and Technology for Development (UNCSTD) next year, some coordination between the demands of the developed and developing world is essential during the months preceding the conference, if it is to have any degree of success. This is the view of one intergovernmental organisation—the Commonwealth Science Council (CSC)—which hopes to make submissions to UNCSTD. The council, which is the scientific arm of the Commonwealth's Secretariat, believes itself to be ideally suited to attempting such coordination. The title of one of its proposed submissions, 'Bridging the implementation gap', reflects this view.

The countries of the Commonwealth make up over a quarter of the United Nations membership. In fact, the Commonwealth could almost be viewed as a United Nations in miniature. The distribution of rich and poor nations can be summarised with reference to two of its members: Canada and Bangladesh. Their views on science and technology are as different as their incomes, a fact which illustrates the difficulties inherent in any claim that there exists a Commonwealth view.

But there is a history of Commonwealth cooperation in science and technology. The very existence of specialist organisations which, under the aegis of the Commonwealth Secretariat, deal with such diverse subjects as geological research, microorganism collections, agricultural science, aeronautics and space research, reflects this. So it seems reasonable to suppose that the CSC will put together a paper which at the very least will document some of the successes and failures of development as witnessed by the Commonwealth.

According to its secretary, Christaen de Laet, the council hopes to prepare a "synoptic overview" of Commonwealth government national papers. De Laet says that the CSC will analyse the framework within which science and technology functions, and consider their application to development, identifying "the contraints imposed by circumstances and the options open at the policy level". This report could constitute another of the submissions to be made to UNCSTD.

'Bridging the information gap' itself does, however, appear to be one of the council's most productive proposals. One of its major considerations will in all likelihood be the obstacles encountered in the dissemination of knowledge within the developing world. One such example, which it will probably discuss, concerns the failure of an indigenous ball-bearing industry in India. In this case, it was the Indian government which had to create a demand for the bearings. All went well, until an American firm offered the government a cheaper product, which it accepted, at which point the indigenous industry collapsed. The CSC will no doubt also consider the state of village agriculture in Malaysia, and the acceptance of alternative energy technology in the Fiji Islands, two projects which are still operating, and which could provide useful case studies for delegates to UNCSTD.

Preparations for UNCSTD are considered by many to be as of much potential value as the conference itself. Some countries, including Papua New Guinea, Zambia and Bangladesh are using the opportunity provided by the preparation of national papers to stimulate discussion on the formulation of their own national science and technology policies. Papua New Guinea, in particular, is preparing a white paper on science and technology policy at the same time as it carries out its work for UNCSTD.

Few people would deny that information is an expensive commodity. Inflation in the West has led to an enormous increase in the cost of scientific journals and books. The repercussions of the price rises have been serious for university and other research departments in Europe and America; throughout the Third World, they have been disastrous. Guyana and Bangladesh in their national papers are likely to place considerable emphasis on the need to have their own scientific library and information services. Countries such as Britain, which pride themselves on the literature they supply to the developing world through bodies such as the British Council would do well to consider this. The current supply is far from adequate, and it is quite clear that there is a growing demand for this category of literature in the developing world. The richer nations ought to be considering how they can meet this need.

Guyana and Bangladesh consider personal contacts to be one of the most effective means of transmitting information. Both countries are likely to suggest that the means be found to increase communication between the scientists of rich and poor nations.

The national papers of Bangladesh and Kenya, and the sub-regional paper of the South Pacific countries will probably consider the 'brain drain' which is acute in the islands of Fiji, in Western Samoa, Papua New Guinea and Bangladesh amongst others. The Kenyan national paper is certain to reflect this but it will put it in a different way by pointing out that university graduates tend to be absorbed by private industry following their training in public corporations—at the taxpayers' expense.

Educational systems never fared well under colonial administrations, which tended to recruit graduates to staff and run the local civil service.

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Students of the arts and social science subjects were in greatest demand; and this was, and continues to be, reflected in the graduates emerging from universities. The proportion of those with any form of technical training remains pitifully low, and many of the developing countries are likely to state this in their papers.

Industrial patents and 'tied aid' are likely to feature prominently in the papers of Kenya, Guyana and Bangladesh. Patents are consuming an ever-increasing proportion of Third World financial resources, which is naturally resented by many. Some are likely to go so far as to say that the high cost of technology transfer is inflated through patents, trade marks and licences.

The UK Ministry of Overseas Development (ODM) has successfully negotiated an increase in its budget by virtually promising it will use the money to buy more technology from British industry. Many developing countries are sceptical, however, one likely objection being that freedom of choice is thus restricted. As for the alternative choices, there remains little information about them in the developing world and what there is does not suffice to provide the country with the means to negotiate favourable deals in a highly competitive and complex world market.

The demand from the developing countries is for more appropriate technology. Technology itself is not enough, however; what is also essential is the 'know-how' of operation and maintenance.

It is the considered opinion of many developing countries that industrial companies are failing to reveal this information. As technology becomes more complex, so the dependence of Third World countries on outside experts increases.

Many of these charges are not being voiced for the first time. The plea for more literature has been made over a number of years. Yet many countries view UNCSTD in a favourable light; preparation for the conference has enabled them to identify and rationalise their own science policies. But UNCSTD will provide the opportunity for a meeting of minds. If the CSC can demonstrate common ground between the nations of the Commonwealth, that in itself will form a valuable contribution. □

Dutch recombinant DNA guidelines to be relaxed

DUTCH guidelines for recombinant DNA research—based on the British and NIH guidelines—are too strict. They are to be reassessed in the light of recent research, and relaxed somewhat within the next few months.

These are some of the conclusions of the second annual report of the Dutch commission of experts concerned with the supervision of work involving genetic manipulation. The commission, formed in 1976 by the Royal Academy of Sciences on the request of the ministers for education and sciences and science policy, pays great attention in the report to recent developments in assessing the hypothetical risks of recombinant DNA research.

The commission, in line with opinion outside the Netherlands, considers it very important to have uniform international guidelines for experiments with recombinant DNA to avoid national differences which would lead to unequal opportunities for researchers of different nationalities. In its report, the commission reaffirms its recommendations to the previous government last year to establish legislation for recombinant DNA research.

Last August, the then minister for health and environmental protection instructed the commission to exercise the utmost caution in coming up with its recommendations. Many thought this indicated a call for a ban on recombinant DNA research, but since then the commission has continued with its work as before. Now 11 projects—all in the NIH P1 category or the Williams' report category I—are going on at three universities (at the end of 1976 there was only one project). In 1977 30 proposals were assessed (against nine in 1976) in eight laboratories within five universities. Industry has plans to become involved but has not begun experiments yet.

The new government, in a statement issued to coincide with the publication of the report, said that research in the two lowest categories can continue —but only if done under the commission's supervision. Supervision, as in the UK, is on the basis of gentlemen's agreements with universities and institutes.

Earlier this month a public discussion was held in Holland with the purpose of informing politicians and members of local and regional governments of the issues associated with recombinant DNA research. About 500 people attended, most of them being scientists, trade unionists and politicians. It was the first time that a forum with the aim of airing a scientific matter in public had been held in Holland.

Casper Schurring

Ariane heads south

A FULL-SCALE model of the fuel assemblies of Ariane, the European Space Agency's satellite launcher currently under development by the French Centre National d'Etudes Spatiales (CNES), left Le Havre for the launch base at Kourou in French Guiana last week. As soon as the model is in position on the launch pad, the CNES will begin testing it for compatibility with facilities at the pad—such as the platform and tower—and for correct functioning. Aerospatiale, the "system integrator," will assess the flight behaviour of the launcher in the local climatic conditions and the effect of vibration during lift-off.

By the beginning of next year, development of all stages and components should be complete. The first qualification launch, which will carry ballast as its payload as well as equipment to measure flight characteristics, is scheduled for June 1979. The subsequent three qualification flights, which will be launched before October 1980, however, will be used to put several satellites into orbit.

Production of the first five operational Arianes was agreed by the ESA member states earlier this year at a total cost of 163 m AU (1 AU= £0.67). Three will be used to launch ESA's Exosat, Marots-B and ECS-1 satellites in 1981. They will be financed out of the relevant ESA programmes. One, which will launch the French Earth observation satellite SPOT will be paid for by France. And the fifth will be kept in reserve and funded by ESA until a payload can be found for it. □

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Proposition 13 threatens US basic research

David Dickson describes fears expressed at a recent meeting of the AAAS on federal research and development

PRESIDENT Carter's much-welcomed boost for basic research in his budget proposals for 1979 is beginning to look like a short-lived affair, faced with demands from tax-payers—in the wake of California's "Proposition 13"—and from economists for a reduction in spending. Already the president has had to appeal to Congressional appropriations committees to respect the integrity of his carefully-structured research and development package, following attempts to subject it to piecemeal cuts.

In the longer term, the administration is facing an uphill battle defending the logic of its position that R & D is an important national priority in need of greater support than it has been receiving in recent years.

President Carter's appeal was made two weeks ago in a letter to the chairmen of the relevant committees and subcommittees. In particular, it was timed to coincide with a meeting at which the Senate appropriations committee was to have considered a proposal to cut the appropriation to the National Science Foundation by $44 million (a cut which was approved by the House last week).

As it turned out, the Senate committee ducked the issue by sticking closely to procedural rules and stating that it would not consider the NSF appropriations until the relevant authorisation bill had been passed by the Senate — which is not expected for a few weeks.

However even if Congress restores the cuts as the president has requested, the problems for basic research are far from over. Mr W. Bowman Cutter, for example, executive associate director of the Office of Management and Budget, had few words of comfort when he spoke last week to a two-day meeting on federal research and development policy organised by the American Association for the Advancement of Science.

Stressing growing concern about the effects of inflation, as well as the president's determination to cut the budget deficit, Mr Cutter said that the 1980 budget was likely to be "the tightest budget in a decade", with cuts across the board in order to make a convincing case for general constraint.

Many at the meeting drew caution from these remarks, as well as from the recent Congressional actions on the 1979 budget requests. "This may be a signal that there is to be a levelling off after one year of attempted build-up," warned Mr William Carey, an ex-White House budget official and now executive secretary of the AAAS.

There was little pessimism in the keynote address given by Dr Frank Press, director of the Office of Science and Technology Policy. Emphasising the need for a better definition of, and greater consensus on, long-term goals for the federal role in R & D, Dr Press said that "there is clearly a good case now for more rational management of the nation's planning and support of its science and technology in the face of their critical relationship to our future".

Dr Press said that during the past year, the administration has initiated new thrusts in areas such as human nutrition, competitive agricultural research grants, climate research and earthquake hazard reduction. "In the coming year, I think we can look forward to many more accomplishments —even in the face of strong challenges and stringent priority-setting," he said.

A central theme of the AAAS meeting—and one that has occupied much of Dr Press' time at OSTP — was the relationship between the declining productivity of US industry and recent decreases in federal support for R&D. In particular, concern has been expressed at industry's apparent shift away from long-range research goals offering the possibility of major technological innovations, towards shorter-term goals concerned largely with product development.

However, while admitting that this process had taken place, few of the industrial representatives at the meeting saw this as a major problem. More relevant obstacles to successful innovation, in their eyes, were the inhospitability of the economic climate, and the uncertainty surrounding future government regulations.

A major implication of this shift in industry to short-term research has been to increase the relative responsibility of the university sector for the conduct of basic research. "Universities still provide the best environment by far for the long-term focus and pluralistic approach so essential to basic research," said Mr Russell Peterson, recently-appointed director of the Office of Technology Assessment.

Yet as a number of speakers pointed out, the universities' current ability to meet this responsibility is far from happy. Much of the 5 per cent increase proposed by the president for basic research over the increase in cost of living would, for example, be needed to replace out-dated equipment.

There was a measure of agreement that more should be done to encourage co-operation between universities and industry in carrying out research projects. Also that policies on patent rights, and on legitimate rates for "overheads" charged on federal research contracts — two current thorns in the side of the administration — should be sorted out in a mutually-acceptable way.

Dr John Sawhill, a previous associate director of OMB and now president of New York University, argued for a system of decentralised grant-making for interdisciplinary research projects of social relevance which would operate in parallel with the existing system, but for which applications would be subject to an internal peer-review within educational institutions.

Congress already sceptical of the peer review process, and is reluctant to dispense funds which cannot readily be accounted for. It does not therefore appear to be in the mood for any such major institutional innovations (a large proportion of the funds which the House has cut from the NSF's budget had been intended for applied research and social science projects).

But without such new initiatives, basic research is left in a relatively weak position. As long as the relationship between basic research and technological development is left an article of faith rather than a clearly-delineated mechanism, it remains vulnerable to the marauding attacks of those seeking quick political victories.

In the present climate, neither industry nor the president's economic advisers are putting much emphasis on the need for federal stimulation of basic research; as Mr Carey pointed out, there is no mention of R&D in the latest annual report of the Council of Economic Advisers.

Furthermore, Congress' piecemeal attempts to limit public expenditure are not merely threatening to hold back isolated pieces of research, but are also undermining the logic of the strategy by which Dr Press put the budget package together.

OMB is about to send out to Government agencies and departments its guidelines for budget requests for the fiscal year 1980. Whether, by the time these have been whittled down into the President's budget statement next January, these will provide a strong case for any further increase in the support of basic research over and above the proposals in this year's budget message remains to be seen. □

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Saudis warm to solar energy

Even Saudi Arabia is searching for new sources of energy. Here **Ziauddin Sardar** reports on the Saudi approach to the sun

SOLAR energy has arrived in Saudi Arabia; and in keeping with the Saudi style, has arrived in a big way.

Two factors are responsible for the current upsurge in solar energy activities in the Kingdom. The first is the swish, aggressive, sales techniques of the French, Swiss and American solar energy salesmen. The Saudi could hardly resist such tactics. But the second is a genuine desire for developing alternative energy sources.

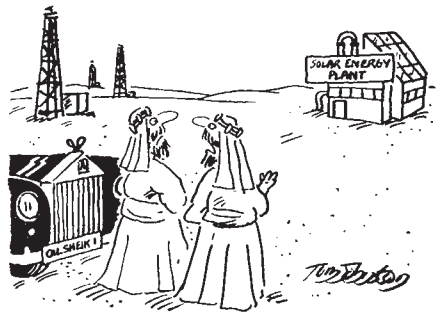
The Saudis well appreciate that oil wells have a finite life; twenty, thirty years at the most. This realisation has been articulated by more than one Saudi minister; and recently it even received royal approval from Crown Prince Fahd. Opening a meeting of the governing board of the Arab Bank for Economic Development in Africa, Prince Fahd said that Saudi Arabia "continues honestly and truthfully to provide the required output of oil and gas because it feels it shares a responsibility with other nations towards the international community. But our feelings of responsibility towards the coming generations in Saudi Arabia require us to look at the situation carefully and to try to balance current requirement with future needs". He went on to say that while it is recognised that oil should be conserved "we are anxious because world consumption of energy is rising at a time when the interest of oil producing countries, as well as of consuming nations, requires stringent measures to cut down consumption and find alternative sources".

Saudi Arabia itself has chosen to back the solar energy alternative, with the accent on achieving solar energy self-sufficiency by the year 2000. Saudi solar energy enthusiasts argue that the Kingdom is even richer in the sun than it is in oil.

Being mostly desert, Saudi Arabia has one of the highest insolations in the world. The Kingdom receives some 3500-4000 hours of sunshine and the average yearly solar intensity is about 550 cal/cm^2/day. It has argued at the recently held first Solar Energy Conference (King Abdulaziz University, Jeddah, 21-23 January) that this energy can be tapped to distill brackish water for drinking and agriculture, generate power, cook food and dry crops. The extreme variations in climate during the day and night can lead to the application of passive cooling during the summer. Geothermal energy in some parts of the Kingdom as well as greenhouse technology can make Saudi Arabia self-sufficient in energy and food.

Probably the single most important outcome of the conference, which was attended by 119 international scientists, was the formation of the Saudi Solar Energy Commission. Lead by Dr Fawaz Alamy, Vice Dean of College of Engineering, King Abdulaziz University, the commission is entrusted with coordination and encouragement of solar energy research in the Kingdom.

Solar research activities in the Kingdom have been pioneered by the University of Riyadh where studies in heating and air-conditioning applications of solar energy have now been conducted for over a decade. Other research activities include solar electronic power generation at the University of Petroleum and Minerals in Dahran; agricultural and industrial applications of solar energy at the King Faisal University, Dammam; and water desalination research at the King Abdulaziz University, in Jeddah.



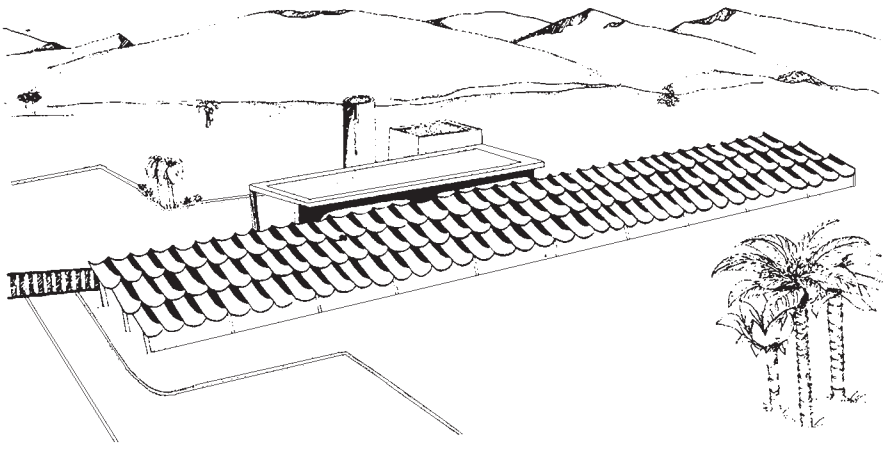
"I hope it's just a mirage"

In addition to the solar energy commission, a Saudi Technology and Research Consulting Centre (STAR) has been set up. It will be largely concerned with the applications of solar energy in air-conditioning, heating, agriculture, electricity generation, weather modification and industry. The centre's first task was to award a grant of five million Australian dollars to Sydney University's Science Foundation for Physics for solar energy research. Prince Nawafibn Abdul Aziz, Chairman of the centre, has already presented a cheque for the initialA$3.5 million to the foundation. The second and final instalment of A$1.5 million will be made in 18 months.

Saudi Arabia has also resolved to encourage solar energy conciousness in other countries. Mr Tarek Al-Akeel of Saudi Arabia's Al-Dariyyah Institute told the delegates at the recently held Soltech exhibition in Bahrain: "The objectives of the Al-Dariyyah Institute are twofold: to encourage the development of solar energy for third world people, who suffer from increases in fossil fuel and food prices, and to encourage industrialised countries to conserve fossil fuel energy so all mankind can be served equally". The Institute has invested US $850,000 in the Terraset School near Washington D.C. which investigates ways of making future buildings energy efficient and solar energy more economic; and US $100,000 in a consciousness-raising cartoon film which will be aired on bought television spots. This is seen as "indirect encouragement" for the conservation of fossil reserves and promotion of solar energy.

In the Kingdom itself, there are four main solar energy projects nearing completion:

- The first reflects the Saudi style of doing things. It is the solar space and water heating system at the Airborne and Physical Training School, King Abdulaziz Military cantonment, Tabuk,



Artist's view of the solar energy plant under construction at King Abdulaziz University, Jeddah

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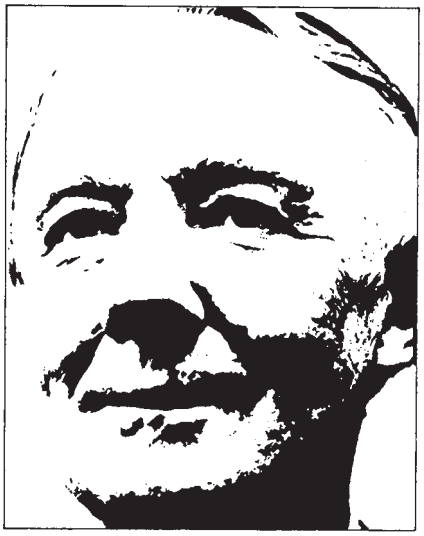
being constructed by Sverdrup and Parcel of St. Louis, Missouri, which is the largest solar energy plant in the world. With some 5,000 m² of collectors, the plant in the system supplies 70% of the total heat load by solar energy. The price tag has a pay back of 20 years; with the replacement of parts included in the cost-analysis. Sverdrup and Parcel claim that the system should be usable for a total of 30 to 35 years. Perhaps!

- The second, a more modest plant, is being developed in Riyadh by Safretes of France. With 6,000 m² of collectors, the system is designed to produce an output of 45 kW of power.
- A similar third plant is being developed by SOTEC of Switzerland for King Abdulaziz University in Jeddah. The project is designed to familiarise students with unconventional seawater desalination method based on solar energy. A solar collector farm will be linked to a multistage flush evaporator for the production of fresh water.
- The fourth project is perhaps the most interesting for it is concerned with the supply of power to small villages in remote places. Normally such villages are supplied by local power stations using diesel engines of sizes varying between 200–2,000 kW. The Kingdom's electricity corporation has decided to replace these stations by thermodynamic conversion solar stations with flat plate collectors. Although it is recognised that solar cells are expensive and any device using them will ordinarily be prohibitive, all considerations of cost have been displaced by the desire to solve the "problems" of the people living in desert areas. The prototype system, which will be used as a research and development centre for solar power plants, is to be designed by Safretes.

It is debatable whether, in the long run, anything lasting can be achieved by such conspicuous projects and flow of capital. The Saudis are quite content with buying off-the-shelf technology—which makes nonsense of all the talk of future self-sufficiency. Not everyone is impressed, however. As one prominent Saudi scientist said at Soltech, "Its all very well to bring this equipment home and play around with it. But don't call that research". □

Self-sufficiency on one-tenth of an acre



KENNETH MELLANBY

WHEN Adam and Eve were driven out of the Garden of Eden, they were told: "By the sweat of thy face shalt thou eat bread", the implication was that they would have to work very hard to obtain enough food to survive. This text from the book of *Genesis* has convinced most people that the life of early man was one of endless toil. They also assume that the same is true for the members of tribes living today whose way of life is usually considered to be primitive.

That this view is generally false is well known to anthropologists, and it is interesting to see the actual figures for hours worked by different communities. Gerald Leach, in his painstaking report 'Energy and food production' shows that hunter-gathers like the ! Kung bushmen of the Kalahari desert in South Africa spend less than a quarter of the 'working week' gathering food, and much of this is hardly a strenuous activity. They are mainly engaged in a leisurely stroll around the large area—over ten square kilometres a head—within which they can pick up their supplies of wild foods.

This idyllic situation was changed when arable crops were introduced by the first true farmers. Much more food was produced, and settlements of a greater density became possible. The cultivators, particularly at times of planting and harvest, had to do more work. Nevertheless, feeding a family with their own produce was hardly a full time job for subsistence farmers. The men at least had long hours for contemplation and recreation, even if they did some of the harder seasonal jobs like clearing secondary bush after a period of fallow. Even the women only worked in the field intermittently, though their elaborate procedures for preparing food kept them busy (and out of mischief) for long hours. Only when the peasants had to produce more food than they could eat, to sell to pay taxes or to buy luxuries, did they begin to work really hard.

Today in most western countries we find substantial numbers of people with the urge to become self sufficient, often as a protest at the rat race of modern civilisation. Some work alone, some in family groups, some in communes. Few succeed, and most seem to demonstrate the truth of the text quoted in my first paragraph. They sweat over their toil, and fail to produce the food they need. This is partly because many lack the necessary training in farming and gardening, and waste much of their effort. But generally failure is the result of trying to do too much. To be really self-sufficient on a few acres of poor ground, when this means not only producing adequate food but also selling produce to provide something approaching an urban standard of living, is not an easy task.

But if they emulate the ! Kung bushmen, who only wished to eat, the situation could be quite different. There should be little difficulty in producing enough food for a healthy diet, though many items we now eat would not be available. If a single man were prepared to obtain three quarters of his food as wheat, he could grow the required amount (200 kg for a yearly ration) on an area of about one twentyfifth of a hectare. Using modern farming techniques this could be planted, fertilised, sprayed and harvested in about half an hour per annum. Even with hand labour, digging with a spade and harvesting with a sickle, one or two weeks of hard work should suffice. He would not have a complete diet, but if he kept a few hens, growing a few more square metres of corn to feed them, allowed his nanny goat to browse on the roadside verges, and did a bit of hunting gathering for wild produce, he could live quite well and still have as much leisure as the fortunate bushman. Trouble would only arise when he began to hanker after the delights of the civilisation he had abandoned.

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What substitutes for oil?

David Spurgeon reports on a forthcoming conference on future sources of organic raw materials

EVER since the OPEC price squeeze drew world attention to the dwindling supplies of oil some five years ago, attention has focused on replacements for petroleum as a fuel. But recently, the vast international chemical industry has begun to concern itself with the prospect of the eventual loss of petroleum as feedstock—and indeed with the entire question of the planet's diminishing supplies of natural resources.

In pursuit of this theme, the International Union of Pure and Applied Chemistry proposed a first World Conference on Future Sources of Organic Raw Materials through its CHEMRAWN programme (Chemical Research Applied to World Needs). Co-sponsored by the Chemical Institute of Canada and the American Chemical Society, the conference is to be held in Toronto, Canada, 10-13 July.

Although most experts do not see petroleum supplies disappearing for at least the next 20 years, oil will inevitably become more and more expensive as supplies diminish. Dr B. W. Rossiter, director of Eastman Kodak Research Laboratories and chairman of the CHEMRAWN committee, said recently that about seven per cent of refined petroleum currently is diverted for chemical use. "Even if petroleum were reserved for chemical use, costs would still rise dramatically. Other sources of carbon-based materials could then become economically more competitive. Consequently it is vital — whatever happens — to learn how to derive useful feedstocks and chemicals from such items as coal. It will also be necessary to perfect recycling methods and to develop entirely renewable resources for forestation, crops, or other parts of the biomass."

The first big push: coal

The general chairman of the conference, Dr W. G. Schneider, president of the National Research Council of Canada, says that the problem is much broader than feedstocks.

"We want to identify alternative resources from a long-term perspective, and identify and share those technologies that are going to be needed to exploit them, particularly small-scale technologies. We're really slanting this whole thing at the turn of the century. What organic raw materials are we going to need at the turn of the century and beyond? We're looking at everything the chemical industry produces — plastics, pharmaceuticals, solvents, agricultural chemicals. A large part of our clothing, food and health require a large amount of chemical processing. We want to examine what the possibilities and the options are."

Some of the options to be considered, besides coal, are wood, plants of various kinds from both land and sea, forest and crop residues, animal and human wastes, liquid fuels such as methanol and ethanol, genetic engineering of microorganisms, anaerobic microbial digestion combined with photosynthesis, and synthetic organic photochemistry.

The first big push for oil substitutes will probably be in the direction of coal. US President Jimmy Carter recognised its importance last year by proposing an increase in the mining and use of coal to 1.2 billion tons annually by 1985 — a proposal that met with broad support from both Congress and the public.

The United States Department of Energy is sponsoring projects in both gasification and liquefaction of coal, and will at the same time explore possibilities of production of chemicals such as ammonia, carbon monoxide, hydrogen and aromatics. Germany also has a government-sponsored programme to produce chemicals from coal.

Germany in fact was the site of development of the first coal liquefaction processes in the 1920s and 30s, and these processes were used on a commercial scale in the second world war. None of them are economic today, however, and the outlook for liquefaction plants that are economically competitive with petroleum as chemical feedstocks is not considered good until the 1990s.

Fortunately, world supplies of coal appear sufficient to meet the need for both fuels and chemical feedstocks for some decades at least. According to a report by Paul Averitt (US Geological Survey Report, 1969, "Coal Resources of the United States", US Geological Survey Bulletin 1275) the world's initial recoverable coal supply ranges between

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about two and eight trillion (10^{12}) tonnes, depending on the depths and thickness of the seams. Of this, 161×10^9 tonnes—or between two and eight per cent — had already been consumed. M. King Hubbert, formerly of the United States Geological Survey, estimates that the peak in the rate of world coal production will probably occur between the years 2100 and 2200.

Brazil takes the lead

One of the most interesting experiments in energy-source substitution is taking place in Brazil, and it may point the way for other countries. Prompted by a massive increase in petroleum imports, (which supplied 13.2% of the country's energy needs in 1940 and 43.6% in 1976), the Brazilian government examined alternatives such as coal, wood, bagasse, ethyl alcohol, vegetable oils, methane and hydrogen; as well as hydro and nuclear power generation, conservation methods, and solar, wind and tidal energy.

In 1975, a National Alcohol Programme was set up to exploit ethanol both as fuel for automotive use (mixed with gasoline), and as a raw material for the chemical industry. Based on future needs, the government forecasts an increase in anhydrous alcohol consumption exclusively for automotive use of 0.73 million m^3 in 1977 to 4.38 million m^3 in 1986.

The methanol is obtained mainly from sugarcane, cassava, babaçú and sugar sorghum, and the government-sponsored programme is searching for new uses for the residues and by-products of the process (stillage, bagasse and yeast). It is also examining problems of distribution of the alcohol.

Production of five million m^3 of anhydrous alcohol is the goal by 1986, which will require an estimated investment of about $2.65 billion. In 1977 the Brazilian government was investing $500 million in the programme but it estimates it will save from $3 to $5 billion in foreign exchange between 1977 and 1986 — no small saving for a developing country.

Offshore seaweed farms

Biomass is another promising alternative to petroleum as a source of both energy and chemical feedstocks. One form being proposed is seaweed, which could be farmed offshore and harvested. Both developed and developing countries could use such a resource to advantage.

One proposal envisages a farm of 100,000 acres growing the alga *Macrocystis pyrifera* 100 miles off the coast of California, which would also have kelp bass and oyster cultivation associated with it. It would produce 42.5 tons of solid material per acre per year, of which 23.8 tons would be volatile. Calculations suggest that such a farm could produce 22.1×10^9 cubic feet of synthetic natural gas annually at $3.65 per cubic foot. (This compares favourably with the present cost of production of gas from coal, $2.47 per cubic foot).

Plant biomass is already being converted to fuel in experiments being carried out in Ghana, Ivory Coast, Indonesia, the Philippines, Brazil and elsewhere in the tropics. The high rates of photosynthesis in these areas suggest that appreciable amounts of energy could be obtained by this means for both domestic needs and for export. The low cost of labour in developing countries makes so-called energy farms even more attractive for them than for developed countries. Crops for such purposes could include sugarcane and cassava, and trees could be farmed or grown in man-made forests.

Wood into plastic

Already-known processes, together with others currently being studied, could be used to transform wood not only into energy but to organic solvents, plastics and synthetic fibres. Lignins are produced in large quantities in waste liquors from pulping processes (20 to 30 million metric tons per year throughout the world), and although they have not been successfully used for other than energy generation, some have proposed fragmenting them into low molecular weight compounds such as vanillin, or into phenols, catechols and benzene derivatives. Others suggest using them as dispersants, emulsion stabilisers or precipitants.

Wood is already used in a small way for production of tar, turpentine, acetic acid, furfural, sugar and other materials. But compared with wood's potential, such production is miniscule. Until recently, it has been cheaper to produce organic chemicals, plastics, synthetic rubber and non-cellulose fibres from oil or natural gas — but with the rising prices of these resources that may soon no longer be the case. It has been estimated that 95% of such materials could be produced from wood.

Both the semi-arid regions of the world and the humid tropics provide possibilities for utilisation of plants and trees. Desert plants often contain a relatively high proportion of hydrocarbons and other energy-rich compounds. They are the source of materials such as fibres, waxes and oils. The guayule plant in Mexico is being grown as a source of rubber. Other plants such as jojoba, mesquite and yucca are being investigated as sources of raw materials. Plant genetics and improved cultivation practices could turn such plants into agricultural crops, greatly increasing their yield.

The humid tropics contain a wide diversity of large trees and plants with great numbers of potentially useful compounds, such as rubber, essential oils and resins. In the temperate zone, silviculture and the conversion of agricultural land masses into biomass factories could yield both energy and chemicals.

The heavier oils will also have to be used as chemical feedstocks in the future: for example, Canada's "tar sands", now the subject of a multi-million dollar project in Alberta. The commercial development of the Canadian oil sands began 50 years ago, and at least 13 pilot or semi-commercial units have been built in an attempt to develop a satisfactory process for extraction of bitumen from surface-mined oil sands. Two commercial plants now are in operation using the hot water extraction process. Great Canadian Oil Sands Ltd., has been in operation since 1969 and has produced between 5,000 and 7,000 tonnes per day of synthetic crude oil for most of that period. Syncrude Canada Limited is in the the process of starting up a 17,000 tonnes-per-day project. Besides the $100 million spent by the end of 1976 on experiments to extract bitumen from the deep tar sands, an additional $200 to $300 million is expected to be spent for this purpose during the next decade.

Planning must start now

Many of the technologies that will have to be developed to make use of the new resources will take 20 years to mature. Since the focus of the Toronto conference is the period beyond the year 2000, this is none too soon to begin planning.

But in addition to technological development, there will be many other problems to face: resource management (particularly when the new uses compete with old ones), environmental impact, and legal and social implications.

Dr Schneider recognised this in the Canadian context: "There's no question that cellulose is going to be an important substance as a primary material and a renewable resource that could be used for energy, chemicals and materials such as plastics, or for conversion to protein or carbohydrates for food. "But we'll have to develop new, fast-growing varieties of trees if we are to use forests for cellulose. And wood supply will be a problem in Canada, for example, if we use it all in pulp and paper production. We have not yet begun to manage our forests." □

correspondence

Western attitude in jeopardy

SIR,—I understand Ziauddin Sardar's frustration (18 May, page 176), but I feel that his views could be damaging in many ways, in particular to the slowly-changing attitude in the West that there is a need for more appropriate development aid. The battle which is bringing about this change in attitude has been fought for many years and in the UK it looks as though it is slowly being won. For example, the UK Ministry of Overseas Development's 1975 White Paper 'More help for the poorest', which set out the case for better aid programmes, is having a positive effect in the West.

Sardar may feel that change is too slow, that it is still muddle-headed and far from appropriate. I would agree with him. But it is up to developing countries themselves to choose the good from the mediocre and to insist on their rights. If they choose to cut themselves off completely they will lose the support which is building up in the West, they will isolate those who have been campaigning for change in Europe and the United States and they will ultimately hold up their own progress.

Not that I think they will. The real answer is not the extreme that Sardar proposes and I am sure many scientists would prefer a middle course. Those in the countries of which I have some knowledge, Vietnam, Mozambique and Tanzania, do want contact with western scientists; and I also know of Egyptian scientists who feel the same.

ALASTAIR HAY

University of Leeds, UK

Lead levels in Birmingham

SIR,—The generally reassuring tenor of Judy Redfearn's article (8 June, page 417), based on the HMSO pollution paper no. 14, largely stems from misleading presentations of three salient aspects of the issue.

Ms Redfearn is wrong to state that there was no significant increase in blood lead levels of people living near the M6–A38 (M) interchange. The HMSO publication quite clearly reports (table 48) an increase of 29% for adult males and 26% for adult females in a group, living 200 m from the centre of the interchange, for whom all confounding factors can be eliminated.

The claim that none of the seven pre-school children with blood lead levels greater than 35 μg% "showed any significant psychological disturbance" is not based on an appropriate assessment and can in no way be coupled with the German, Canadian and American studies which are mentioned later in the article.

The misleading implication that tooth lead levels measured by expert analysts can give results that vary as widely as those found by the inexpert for concentrations of lead in blood is inexcusable; more especially so when no recognition is made of the fact that there is an important analytical advantage afforded by the approximately one hundred-fold higher concentration of lead in a tooth than in its blood-supply.

Instead of portraying the interchange with the legend "Spaghetti junction: a clean bill of health", perhaps a better perspective would be provided by setting the mean blood lead concentrations for males (28.8 μg%) and females (23.2 μg%) (table 49, HMSO pollution paper no. 14) living nearby against the natural blood lead level of 0.25 μg% calculated by Patterson in 1965.

R. STEPHENS

University of Birmingham, UK

Saccharin: the risks and benefits

SIR,—I am writing in reply to the letter by A. B. Miller and G. R. Howe (4 May, page 8) objecting to my paper on "Saccharin: the risks and benefits" (9 February, page 492). The estimate of 1,200 extra bladder cancers per year if each US citizen were to ingest one diet drink per day was not mine; it was given by the US Food and Drug Administration as the basis for their policy of attempting to ban saccharin. My paper simply accepted their estimate of the risk, compared it with the risk of ingesting an equivalent amount of sugar, and showed that the latter risk was 100 times greater for a person who is as much as 10% overweight.

Since the average American is about 12% overweight (*Metropolitan Life Ins. Co. Stat. Bul.*, January 1977), it would seem to be highly beneficial to the public if the food processing industry were to substitute saccharin for sugar. The point that Miller and Howe miss is that sugar is also "a potentially hazardous substance", much more hazardous than saccharin even by their estimates.

BERNARD L. COHEN

University of Pittsburgh, USA

Burying high level wastes

SIR,—R. M. Macintyre (Burying high-level wastes, 16 February, page 605), states that geochronologic K–Ar data argue against the suitability of bedded salt deposits for the burial of high-level waste. It is disturbing to see such generalisations made with data which are improperly interpreted.

It is well-recognised that sylvites (KCl) yield low K–Ar dates. The reason for radiogenic ^{40}Ar loss from evaporite minerals may be dissolution (as Macintyre proposes) or diffusion (Dalrymple and Lanphere, *Potassium–Argon Dating*, W. H. Freeman and Company, San Francisco, 1969). Not considered in Macintyre's correspondence are the following data:

- some sylvites do yield K–Ca ages consistent with their age of formation (Polevaya *et al.*, *Geochemistry*, **8**, 897-906, 1958), thus demonstrating that while an inert gas such as ^{40}Ar can be lost from sylvite, a solid species like radiogenic ^{40}Ca is retained in the sylvite structure.
- K–Ar studies of various K-bearing minerals from the Los Medanos area near Carlsbad, New Mexico (Isochron/West, **6**, 37, 1973) show that sylvites yield anomalously low dates of 18 ± 8 to 74 ± 8 my. A langbeinite yields 245 ± 10 my, a date corresponding to the late Permian (the time of deposition). Two samples of mixed sylvite-langbeinite yield 137 ± 8 my and 147 ± 10 my. Certainly all three dates for langbeinite and langbeinite-sylvite argue against "recent" recrystallisation in these deposits.

In support of the Waste Isolation Pilot Plant project, the Los Medanos area of south-eastern New Mexico (USA) is being subjected to intensive study including geochronology to assess the stability of the bedded evaporites since the Late Permian. Fluid inclusions studies of Roedder have identified nonrecrystallised Los Medanos salt. Certainly there are no data to substantiate ". . . percolating groundwaters . . .". Salt diapirism in subhorizontal salt beds 700 m deep, such as at Los Medanos, has adequately been discussed by Gera (*Geological Society of America Bulletin*, **83**, 3551-3574, 1972).

First results of an ongoing Rb–Sr geochronological study conducted by Brookins and others show that a Rb–Sr isochron based on water-soluble fractions of evaporite minerals and authigenic clay minerals yields a date of 204 my with an initial ^{87}Sr/^{86}Sr of 0.7137. This data is interpreted as a minimum since independent data from Ca-rich phases indicate an initial ^{87}Sr/^{86}Sr ratio of 0.7081 ± 0.0007. Polyhalite and sylvite, properly identified as recrystallisation products, must have formed early in the diagenetic history of the evaporites, else the Rb–Sr dates for such samples would be characterised by very young dates and probably much higher initial ^{87}Sr/^{86}Sr ratios.

Finally, it is unreasonable to assume that the escape behaviour of argon should be representative of all radiogenic daughter products. Strontium, which is also an important mobile constitutent of radioactive waste, has certainly been retained in the evaporites for 2×10^8 years. The impurities such as trioctahedral clays in Los Medanos rock salt have also been found to preferentially absorb from aqueous solution actinides present in waste.

D. G. BROOKINS
J. K. REGISTER
M. E. REGISTER

University of New Mexico, Albuquerque, USA

S. J. LAMBERT

Sandia Laboratories, Albuquerque, New Mexico, USA

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news and views

UPSI-DESY

from David J. Miller

THE Υ (upsilon) particle was first discovered last summer (Herb *et al. Phys. Rev. Lett.* **39**, 252; 1977). Like the ψ/J particle it was found first in proton–proton collisions, it was narrow—within the resolution of the experiment—and it decayed into a pair of leptons (muon and antimuon or electron and positron). Unlike the ψ its properties were not immediately checked by seeing it in electron–positron annihilations at a colliding-beam machine. The problem was its mass. At 3.1 GeV/c^2 the ψ lay within the immediately accessible range of three electron–positron machines which were immediately able to adjust their beam energy to find the new particle. But the Υ and its two excited states the Υ′ and Υ″ are in the region from 9.4 to 10.4 GeV/c^2. Last summer it seemed that the new Cornell University accelerator would be the first machine to reach the GeV/c^2 region in electron–positron collisions—sometime in mid-1978. Now the race to the Υ has been won, but not by Cornell.

At the DESY laboratory in Hamburg the accelerator engineers have rapidly rebuilt the 'DORIS' electron–positron storage rings, changing them from a double to a single ring, accelerating only one bunch of each type of particle, and achieving a new peak energy of almost 5 GeV in each beam. This has allowed them to scan the collision energy up towards 10 GeV. As the beam energies were raised through the Υ region, physicists measured the rate of production of strongly-interacting particles, detecting them in the two well tried devices called 'DASP' and 'PLUTO' which have been used for much of the recent work at DESY on ψs, charm and the τ lepton.

In two papers submitted to *Physics Letters* (Darden *et al.*, DESY, Dortmund, Heidelberg, Lund collaboration with DASP; Berget *et al.*, AAchen, DESY, Hamburg, Siegen, Wuppertal collaboration with PLUTO) both groups report a clear and sharp peak in hadron production centred at 9.46 GeV. The width of the effect is consistent with the energy resolution of the storage-ring (±8 MeV) and the integrated rate gives a partial-width for Υ to electron-positron of 1.3±0.4 keV, assuming that electron–positron is not a major decay mode. They have not yet scanned the energy through the Υ′ at 10.0 GeV or the Υ″ at 10.4 GeV. (Rumour has it that they will leave these to the new storage-ring PETRA which is expected to work at DESY in July, some months ahead of schedule).

The similarities between the ψ family and the Υs are made even closer by this result. Apparently the Υ is a hadron as it decays to hadrons and has been produced by hadron collisions (proton–proton), but it is extremely narrow for such a massive particle. That is, through the uncertainty principle, it has a surprisingly long life for a particle which has available so many different decay possibilities to lighter objects such as mesons, proton–antiproton and so on. The standard explanation of the narrowness of the ψ is that it is a low-lying state of 'charmonium', being composed of one charmed quark and one charmed antiquark, bound together by a strong attractive force due to the exchange of gluons. In the same way an electron and a positron may be bound by photon exchange to form positronium. For the ψ a phenomenological rule, the Zweig rule, is invoked to inhibit all hadronic processes which do not preserve into the final state those quarks which were present in the initial state. The lightest particles which contain single charmed quarks are the D mesons, but their mass is more than half the mass of the ψ, or even of the heavier excited state the ψ′(3.7), so the ψ and ψ′ cannot decay to a $D\bar{D}$ (D and anti-D) pair. They can only decay to non-charmed final states in violation of the Zweig rule, giving long lifetimes and narrow widths. All excited states of charmonium above twice the mass of the D are broad and decay rapidly to final states containing DD, but there are only narrow states below the ψ′ mass.

Given the success of the charmonium picture of the ψ it is natural to interpret the Υ family in the same way. One or more new quarks are suggested, with the Zweig rule inhibiting rapid Υ decay into charmed or normal final states. The value for the Υ to electron–positron width reported from DESY supports this picture. It corresponds exactly to what would be expected if the Υ is a low-lying state of a new 'onium' formed from a quark and an anti-quark of charge 1/3 (the charmed quark has charge 2/3 like the common 'up' or 'proton' quark. The common 'down' or 'neutron' quark and the strange quark have charge −1/3). The most popular approach to the Υ is to introduce a pair of new heavy quarks called 'top' and 'bottom'. If the Υ is 'bottomonium' then it is expected that a more massive equivalent of the D mesons will be found at a little over 5.2 GeV/c^2—just above half the mass of the Υ″. Since the D is often described as 'naked charm', compared with the 'hidden charm' of the ψ, then these newer more massive mesons will offer our first view of 'naked bottom'.

Although the conventional approach through the Zweig rule and a minimum number of new quarks accounts for the major features of the Υ and ψ spectra so far, there are many theorists who feel unhappy about the *ad hoc* nature of many of the arguments involved. A host of alternative schemes exists, and it will be the job of the storage-ring physicists to explore the whole range of properties of the Υs in order to pin down exactly what kind of new quarks we are dealing with. □

David J. Miller is in the Department of Physics and Astronomy, University College, London.

0028–0836/78/0273—0705$01.00

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Is prostacyclin a circulating anti-coagulant?

from C. T. Dollery and C. N. Hensby

LUNG tissue from numerous animal species, including man, has been shown to be capable of synthesising and releasing a variety of prostaglandins (Piper & Vane *Ann. N.Y. Acad. Sci.* **180,** 363; 1971). Furthermore, metabolic transformation in the lung rapidly inactivates these prostaglandins (Ferreira & Vane *Nature* **216,** 868; 1973). It seemed unlikely, therefore, that the naturally occurring prostaglandins would be released from the lung in sufficient quantities to act as 'circulating hormones'. One exception to which the term 'circulating hormone' could be applied was the evidence in many animal species (but not man) that prostaglandin $F_{2\alpha}$ was the uterine leuteolytic hormone (for references see Horton & Poyser *Physiol. Rev.* **56,** 959; 1976). In this instance, however, prostaglandin $F_{2\alpha}$ cannot be considered as a genuine circulating hormone, as a complicated transfer mechanism has evolved, enabling $PGF_{2\alpha}$ to pass directly from the uterine vein into the ovarian artery, thereby bypassing the lung inactivation process. With the discovery that prostacyclin, the major product of arachidonic acid metabolism produced by arteries and veins (Moncada, Higgs & Vane *Lancet* **i,** 18; 1977) could pass through the lung circulation unchanged (Armstrong *et al. Brit. J. Pharmac.* **62,** 125; 1978) the possibility that prostacyclin could function as a circulating hormone had to be given serious consideration. In this issue of *Nature,* two independent groups, Gryglewski's from Poland (page 765) and Vane's from England (page 767) present evidence to suggest that lung vascular tissue continuously produces and releases prostacyclin *in vivo* and that this substance acts as a circulating anti-thrombotic agent especially on the arterial side of the circulation. Other evidence has been presented to support this theory (Moncada & Korbutt *Lancet* **i,** 1286; 1978). These authors suggest that prostacyclin increases platelet cyclic AMP thereby decreasing platelet aggregability. Whether the prostacyclin liberated from the pulmonary circulation is a 'spill over' phenomenon rather than a fully functioning anticoagulant mechanism has still to be determined. However, the results obtained by Moncada and Korbutt suggest that this is the case—at least in the rabbit. Recently Boot and his colleagues (*Int. Arch. appl. Immun.* **57,** 159; 1978) have confirmed that prostacyclin is the major product of arachidonic acid formed in the pulmonary vascular bed. The extent to which prostacyclin has a local role in the lung in regulating the resistance of the pulmonary vascular bed also remains to be established but it is an aspect which ought not to be forgotten. These workers have also shown that the lung can metabolise prostacyclin after it has been hydrated to 6-keto $PGF_{1\alpha}$ so the lung can both form and inactivate prostacyclin.

Factors that modify prostacyclin production in the lung may be of some importance in relation to lung pathology and intravascular thrombosis. Gryglewski and his colleagues suggest that noxious stimuli such as cigarette smoking may reduce formation of prostacyclin and increase the risk of thrombosis. Interestingly, Boot and his colleagues found that successive immunological challenge decreased the production of prostacyclin whereas formation of thromboxane A_2 was increased. This too may have implications for pathology.

The implications of recent research on circulating prostacyclin for clinical medicine may be substantial. Formation of blood clots in veins seems to be a much more common event than in arteries. There may be several reasons for this; a lower velocity of flow in veins must be one, but the presence of higher concentrations of prostacyclin in arterial blood may turn out to be more important. Extension of blood clots on the venous side of the circulation also seems to be more common than on the arterial and a possible explanation may again lie in the higher levels of prostacyclin. Ever since the discovery of the highly potent platelet-aggregating activity of thromboxane A_2 released from platelets, what limits the chain reaction of intravascular thrombosis has been a puzzle. In theory it seemed that aggregation of more platelets to a thrombus would release yet more thromboxane and aggregate more platelets until either all the platelets had been sequestered or the whole circulation had thrombosed. The presence of prostacyclin in the blood would act as a negative feedback mechanism limiting the spread of the clotting process back into the flowing blood.

Interest is bound to shift towards pharmacological means of manipulating the balance between formation of thromboxanes and prostacyclin as a more physiological means of controlling intravascular coagulation. It also seems possible that either synthetic prostacyclins with greater stability than the natural compound or drugs which selectively inhibit thromboxane synthetase may be useful as anti-coagulant drugs, for example in extracorporeal circulation through artificial kidneys or oxygenators. For long it seemed that prostaglandin research might remain as an exciting biological discovery which had very limited applications to human disease. With the discovery of the thromboxanes and prostacyclins it seems destined to return to the centre of the stage. □

C. T. Dollery is Head of the Department of Clinical Pharmacology and C. N. Hensby is a member of the research staff of the Department of Clinical Pharmacology, Royal Postgraduate Medical School, Hammersmith Hospital, London.

A new interaction for leptons?

from F. E. Close

SOME recent data from Gargamelle (*News and Views* **273,** 98; 1978) have raised a question mark over the Weinberg-Salam model which is the simplest candidate for unifying the weak and electromagnetic interactions. An experimental study was made of the elastic scattering of muon neutrinos on atomic electrons. Twelve events were found where only two had been expected according to the model.

Any player of roulette will know that it is not uncommon for one to win a small amount when 4 or 5 successive reds emerge. A run of 50 successives would be a clear indication that some external agent was at work. The immediate concern in the Gargamelle data is to have more of it to see if the present result is just a statistical fluctuation or whether it heralds a genuine effect. It does seem that statistics may turn out to be the culprit. A recent seminar at Fermilab has been reported as showing that in an analogous experiment there, six events were seen where six were expected. These data are still being analysed.

The interesting theoretical question therefore remains for the moment: what must we do to the Weinberg-Salam model if the effect is genuine?

Clearly, many ideas will be put

F. E. Close works at the Rutherford Laboratory.

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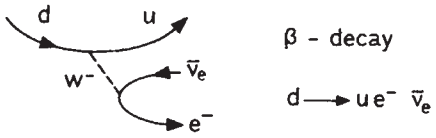

forward. The first of these is by a collaboration involving Salam (see this issue of *Nature* page 736) who report in a footnote that Weinberg has also independently worked out a similar idea. In a nutshell: just as the quarks feel the strong interaction but leptons do not, so the 'symmetry' is restored by postulating that leptons feel a new interaction which the quarks do not. This is the origin of the excess of the (purely leptonic) Gargamelle events.

Leptons, quarks and colour

The salient features of the original Weinberg-Salam model were as follows. The elementary particles are of two types—leptons and quarks. Both leptons and quarks experience the electromagnetic and weak forces. The quarks but not the leptons can also experience the strong (nuclear) force.

In the phenomena experienced in our everyday world we meet just two leptons—the electron in atoms and its ghostly brother the neutrino in radioactivity and the burning of the Sun, and two flavours of quark—up and down—which build up the neutron, proton and ultimately nuclei. These quarks and leptons form the 'first generation of elementary fermions'.

In the weak interaction of radioactivity it has been known for many years that the neutrino turns into an electron or that an up quark transmutes into a down. The process of β-decay is the best known example (see figure). The electrical charge is changed at the lepton vertex but is exactly balanced by an equal but opposite change at the quark vertex. This is an example of a 'charge changing weak interaction'.

A prediction of the Weinberg-Salam model was that neutral weak interactions should also exist where a neutrino stays a neutrino and an up (or down) quark remains an up (down) quark. Such an interaction was subsequently found (*Nature* **245,** 119; 1973; **249,** 211; **250,** 186; 1974) with properties consistent with the predictions of the model.

There will also be a weak neutral current where an electron turns into an electron. This will be additional to the familiar electromagnetic current of the electron. The latter is most familiar in atomic physics. The presence of the weak neutral current will give rise to interesting new phenomena such as parity violation in atomic physics (see *News and Views* **264,** 505; 1976 and the figure in *News and Views* **273,** 99; 1978).

It seems that to make the model fully self-consistent theoretically at extremely high energies, the total amount of electrical charge of the elementary fermions must be zero. The leptons have a total of -1 (due to the electron; the neutrino is electrically neutral). The up and down quarks have charges of $\frac{2}{3}$ and $-\frac{1}{3}$ respectively yielding a total quark charge of only $\frac{1}{3}$ whereas a net $+1$ is needed to compensate for the leptons net -1. Fortunately there is evidence from various independent data that suggest

Virion RNA can be used to study nuclear processing of messenger RNA

from R. Weiss

WITH the discovery of splicing of mRNA in eukaryotes there has been renewed interest and excitement in the nuclear processing of mRNA from larger transcripts. One problem in studying the editing mechanisms of mRNA production is the difficulty of obtaining substantial amounts of stable and pure precursor RNA for use in processing experiments. On page 779 of this issue of *Nature*, Stacey and Hanafusa describe a promising system for such studies. The 35S genomic subunits of virion RNA of avian RNA tumour viruses resemble mRNA in structure and can be translated *in vitro* to synthesise the product, Pr76, of the *gag* gene which is situated at the 5′ end of the RNA molecule. The *env* gene, coding for virion envelope glycoproteins is located nearer the 3′ end of the 35S genomic RNA and is not translated efficiently *in vitro*. In the cytoplasm of infected cells, however, a 21S mRNA has been isolated which carries the genetic sequences of the *env* gene (Hayward *J. Virol.* **24,** 47; 1977). The subgenomic 21S mRNA seems to be a spliced message as it includes a 5′ terminal leader sequence juxtaposed with sequences derived from the 3′ half of the virion molecule (Mellon & Duesberg *Nature* **270,** 631; 1977; Weiss, Varmus & Bishop *Cell* **12,** 983; 1977).

Stacey and Hanafusa have used a sensitive means of detecting synthesis of small amounts of envelope glycoprotein translated from the 35S genomic RNA of an avian leukosis virus, RAV-2, microinjected into cells. The cells used for microinjection are already infected with a strain of Rous sarcoma virus (RSV) that is defective in envelope glycoprotein. If glycoprotein is synthesised by translation of the RAV-2 RNA, the defective RSV is complemented and infectious RSV particles are produced. Using this technique they show that 35S genomic RNA inoculated directly into the nucleus results in rescue of the defective RSV, whereas inoculation of the same RNA preparation into the cytoplasm barely yields any infectious RSV. As a positive control, cytoplasmic inoculation of 21S message causes the most efficient RSV rescue. Stacey and Hanafusa have not been able to follow the fate of the microinjected RNA directly, but on the basis of these data, it seems that nuclear processing of the inoculated virion RNA takes place, yielding an efficient *env* messenger molecule which is then transported to the cytoplasm and is translated into the glycoprotein precursor. The supposition is that the natural 21S *env* mRNA is produced by the same mechanism, rather than by a direct transcription of subgenomic mRNA from the DNA provirus. Nevertheless, a considerable amount of 35S RNA is exported intact to the cytoplasm to serve as mRNA for *gag* proteins and polymerase as well as for genomic RNA to be packaged into virions. The most important result of the evidence for nuclear processing is, as the authors note, that virion RNA can be used as a readily obtainable 'nuclear' precursor for mRNA. This, one hopes, will open the way for testing mRNA processing *in vitro* using nuclear extracts which may lead to the identification of splicing enzymes.

It should be pointed out, however, that in a previous study using the same RSV rescue technique, Murphy and Ozanne (in *Avian RNA tumor viruses,* (eds Barlatti & de Giuli-Morghen) Piccin Medical Books, Padua, 1978) showed that 35S genomic RNA introduced into cells in culture by treament with DEAE-dextran resulted in glycoprotein production (as well as *gag* proteins) almost as efficiently as Stacey and Hanafusa obtained by nuclear microinjection. Again, the fate of the 'transfected' RNA could not be followed directly, but the discrepancy in translation of virion RNA introduced into the cytoplasm by microinjection or by transfection requires explanation.

R. Weiss works at the Imperial Cancer Research Fund Laboratories, London.

0028–0836/78/0273—0707$01.00

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that the up and down flavours of quark actually each occur in any of three 'colours', red, blue and green. Taking this into account the total quark net charge is $\frac{1}{3}$ for each colour, hence +1 in all. The total charge of the elementary fermions is therefore zero as required. It therefore seems that we have found all the ingredients for a fully satisfactory theory of weak and electromagnetic interactions.

This colour degree of freedom has been exploited to make a theory of the strong interaction known as quantum chromodynamics where the colour is a new type of charge (analogous to electrical charge and its role in quantum electrodynamics). The fact that quarks have colour but leptons do not is the origin of the quarks experiencing the strong interaction. Specifically the quarks can couple to eight coloured gluons which are the carriers of the strong force, rather as particles with electrical charge couple to the photon, carrier of the electromagnetic force. Uncharged particles do not feel the electromagnetic force; uncoloured particles (like leptons) do not feel the strong force.

With all of this beautiful self-consistency, why did Nature choose to repeat itself by invoking a second generation of fermions, namely a muon and another neutrino in the lepton world with a strange and charmed quark (each in three colours again) in the quark world?

The answer to this is not known. It is worth pointing out that the Weinberg-Salam model plus QCD is as applicable to the second generation as it is to the first. Indeed in 1973 when the neutral currents were first seen the charm quantum number had not been found. Glashow and collaborators (*Phys. Rev.* **D2,** 1285; 1970) had recognised the relevance of the doublet pattern of quarks and predicted that charm must exist. The discovery of J/ψ in November 1974 (see *News and Views* **252,** 438) and of charmed particles in 1976 (see *News and Views* **262,** 537) dramatically provided the missing link. Charm exists and apparently is the required partner of the strange quark.

Neutral current interactions can take place between the leptons of the second generation and the leptons or quarks of the first. Such interactions have been observed and the elastic scattering of muon neutrinos from up and down quarks seems to agree with the model predictions. The surprising result from Gargamelle suggests that scattering from first generation leptons contains some new ingredient.

Colour for leptons?

An excess of purely neutral current events has been found compared with the predictions of the model. This suggests that there may be a purely leptonic neutral current in Nature in addition to the one already in the model. To this end Pati, Rajpoot, Salam and Strathdee (this issue of *Nature,* page 736) have invented the idea of leptonic colour.

If there are two leptonic colours then the neutrino is red or blue (these are what we have previously called the electron neutrino and muon neutrino). Similarly the electron is red or blue (what were previously called the electron and muon). These two colours form an SU(2) group of leptonic colour analogous to the SU(3) group of quark colour. Just as the strong interactions are generated by the latter, so will a new interaction be generated by the leptonic colour. Precisely for the same reasons that strong interactions are only experienced by particles containing quark colour so will the new interaction be felt by particles containing leptonic colour. Hence this new interaction will not contribute to neutrino-quark scattering (the quarks do not have leptonic colour) but will contribute to the purely leptonic neutrino-electron scattering and is hypothesised to be responsible for the excess of such events at Gargamelle.

What is amusing is that a third generation of leptons and quarks is probably now being uncovered (see *News and Views* **271,** 406; 1978). Hence there are three leptonic colours and the gauge group is therefore SU(3) for leptonic colour. We know already that the quark colour gauge group is SU(3)—is this a coincidence or profound?

The essential difference between the lepton and quark colours is that the gluons of quark colour are massless while those of leptonic colour are massive. Hence this may be why the red, blue and green leptons (electron, muon, tau and their three neutrinos) have been seen but the quarks (red up, blue up, green up and so on) have not. Why this should be so is an open question.

One apparent consequence of the model seems to be the necessary transmutation of red leptons into blue. In more familiar language this means muon turns into electron. A rate for this has not been discussed. However, predictions are made for other weak neutral current processes, in particular the rates for $\bar{\nu}_e e$ and $\bar{\nu}_\mu e$ scattering will be modified relative to the predictions of the old model. These will be interesting quantities to study experimentally to see if a new leptonic force exists. □

DNA repair mechanisms

from R. B. Painter

THE DNA in living organisms is subjected to an impressive variety of hazardous chemicals and radiations that can alter its structure. Fortunately repair schemes have evolved to reverse or nullify the deleterious actions of these agents on the essential genetic material. The importance of DNA repair in man is illustrated dramatically by the hereditary disease, xeroderma pigmentosum (XP), which involves a deficiency in the repair of certain sunlight-induced damage to DNA. Patients with this disease inevitably develop skin cancer as a consequence of several minutes exposure to sunlight. Repair has also been implicated in differentiation and in recent theories on ageing.

One of the paradoxes in the field of DNA repair is that more is known about repair pathways than about the lesions on which they operate. At a recent international workshop on DNA repair* P. Cerutti (University of Florida) appealed for a general classification of repair modes based on the kinds of damage induced rather than the agent causing the damage. He pointed out that benzo(a)pyrene, 4-nitroquinoline oxide, and mitomycin C act by both direct and indirect mechanisms as do many other agents. The spectrum of indirect damage, predominantly due to OH radicals formed in water, is similar for a number of agents and therefore can be studied in a general way with a single inducing agent. A.J. Clark (University of California, Berkeley) also called for a new system for classification of repair, his basis being the status of the DNA in terms of its replication. Repair in unreplicated and completely replicated DNA would be combined under the label 'extra-replication repair' while that occurring near replicating forks would be called 'intra-replication repair'. This class, also termed daughter strand repair, would include the processes of recombination, bypass replication, and gap-filling. Clark proposed that incision-promoted recombination excision as well as 'classical' excision-repair may be active in completely replicated DNA. He also postulated an intra-replication repair mechanism called copy choice excision. This is the

*ICN-UCLA Winter Symposium Keystone, Colorado February 19-24, 1978. Organised by P. C. Hanawalt and E. C. Friedberg.

0028–0836/78/0273—0708$01.00

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first time that the copy choice mechanism has surfaced since the failure many years ago of copy choice models for high negative interference in bacteriophages. Clark also made the very interesting proposal that since primitive organisms evolved in sunlight and that pyrimidine dimers must therefore have been produced in their DNA, bypass mechanisms may have evolved first and excision-repair was an added bonus appearing later.

Several investigators showed that if DNA is allowed to replicate for quite a while after ultraviolet irradiation there is an almost complete lack of evidence for recombination, in contradiction to earlier studies on post-replication repair in mammalian cells. J. Cleaver (University of California, San Francisco) and A. R. Lehmann (University of Sussex) both emphasised the importance of knowing the details of normal DNA replication in order to understand what happens when the growing point approaches a lesion. Cleaver, in particular, emphasised the coupling of replication with repair and pointed out the necessity for measuring DNA replication parameters, such as replicon size and fork displacement rates, at the same time that repair measurements are made.

The dilemma faced by scientists studying post-replication repair in mammalian cells is probably not much greater than that faced by investigators of excision repair in prokaryotes, which is generally believed to be a much simpler system. Even the question of whether ATP is required for the incision step of excision-repair in bacteria was debated at this meeting. While L. Grossman (Johns Hopkins University) presented evidence that the *uvrA* plus *uvrB* gene products do not require ATP for endonucleolytic activity, E. Seeberg (Toxicology Division, Kjeller, Norway), using an *in vitro* complementation assay, found that their combination with the *uvrC* gene products results in ATP-dependent nicking of dimer-containing DNA. Even though the exact nature of the incision step of excision repair occurring in *E. coli* remains in doubt, it is clear from the paper of P. Hanawalt (Stanford University) that both the excision and resynthesis steps are primarily performed by the abundant polymerase I. When polymerases II or III carry out repair synthesis, the patch size is larger and the likelihood of error becomes greater.

T. Lindahl (University of Götenborg, Sweden) and others have shown that repair of some lesions that involve only a single base (base excision repair) is generally different from the pathway for pyrimidine dimers (nucleotide excision repair). For these mono-adducts (and for uracil in DNA) the base is clipped off the deoxyribose by a DNA glycosylase, leaving an AP (apurinic or apyrimidinic) site. E. Friedberg (Stanford University) discussed the nearly ubiquitous distribution of these enzymes as well as another class of enzymes probably involved in base excision repair, the AP endonucleases, which incise DNA at sites of base loss. S. Linn (University of California Berkeley) presented fascinating preliminary evidence for an 'insertase', which simply places the correct purine back into sites of purine loss.

Much excitement and controversy has arisen over inducible repair systems. First clearly identified in bacteria, it is now apparent that inducible repair schemes are extremely complex and may also exist in mammalian cells. The infamous protein X, first described in *E. coli* by L. Gudas and A. Pardee (Princeton University), now seems to be the inducible product of the *recA* gene. This interesting protein seems to have at least two functions as outlined by P. Emerson (University of Newcastle-upon-Tyne). One is an endopeptidase controlling its own formation. Combined with an effector molecule the *recA* product removes the repressor for its own synthesis. It also binds to single-strand DNA; this is possibly its role in recombination. Inducible repair does not seem to be limited to error-prone systems, however. P. Shendel (National Institute for Medical Research, Mill Hill) described an inducible error-free pathway in *E. coli* which has been called adaptation. Induction of this repair system renders the organism less susceptible to the toxic and mutagenic effects of certain alkylating agents.

Since the discovery by J. Cleaver 10 years ago that patients with XP are defective in excision repair, interest has increased in this and other genetic diseases that may have their basis in defective repair. D. Bootsma (Erasmus University, Rotterdam) reported seven complementation groups in XP, two discovered within the past year. These are in addition to the so-called XP variant, the condition in which the clinical disease exhibits no excision repair defect. Exciting evidence that may soon shed light on the relationship of dimers to cancer in XP was described by B. Sutherland (Brookhaven Laboratories, New York). Transferrable clones grew in soft agar after exposure of human diploid cells to ultraviolet light. The direct induction of human malignant cells by pyrimidine dimers may have been demonstrated in this experiment.

A second disease that exhibits a defect in DNA repair is ataxia telangiectasia. M. Paterson (Chalk River, Canada) showed that cells from persons with this disease are X-ray sensitive; some patients, but not all, show a defect in repair replication after X irradiation. Paterson presented evidence for several complementation groups in this disease and also showed that some heterozygotes are moderately sensitive to ionising radiation.

A third disease implicating defective repair, discussed by M. Sasaki (Tokyo University), is Fanconi's anaemia, a disease of progressive hypoplastic pancytopenia with associated leukaemia, malignant neoplasms, and increased chromosomal fragility. The defect seems to be in excision repair of interstrand crosslinks. J. German (New York Blood Center) presented evidence that Bloom's syndrome and Cockayne's syndrome may also be associated with repair defects, although the particular defect has yet to be identified. Dyskeratosis congenita is a relatively new candidate for a repair defect; like Bloom's syndrome it shows increased chromosome fragility.

Perhaps the most provocative new lead in mammalian DNA repair processes was the demonstration by R. Benjamin (Harvard University), M. Jacobson (North Texas State University, Texas) and R. Skidmore (University of Sussex, England), that the rate of synthesis of poly ADP-ribose increases during repair of DNA damage induced by X irradiation or alkylating agents. Thus, it seems that this mysterious macromolecule may have a role in DNA repair. □

R. B. Painter is Professor of Microbiology, Laboratory of Radiobiology, University of California, San Francisco.

Rings, MAPs and microtubules

from Roy Burns

SINCE procedures for repolymerising microtubules *in vitro* were first defined about 5 years ago there have been numerous reports characterising the kinetics of reassembly and describing tubulin oligomers which may or may not be intermediates in microtubule assembly. These oligomers have been examined by biochemical techniques (ultracentrifugation and gel exclusion chromatography) and ultrastructurally, and have been described in various laboratories as single rings, double rings, disks, stacks of disks, and helices. It is also clear that the oligomers found by different laboratories

Roy Burns is a Lecturer in Biophysics in the Department of Physics, Imperial College of Science and Technology, London.

0028–0836/78/0273—0709$01.00

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using different procedures to cycle the microtubule protein, notably whether glycerol is used or not, differ from each other. Furthermore the formation of some of these oligomers requires the presence of non-tubulin proteins, called microtubule associated proteins (MAPs), and different purification procedures select for different MAPs, so that some laboratories enrich for a pair of high molecular weight proteins (HMWs) while others enrich for proteins of molecular weights (58,000–70,000 daltons) which have been variously termed tau.

Some of this confusion has finally been clarified by an elegant series of experiments by Borisy and his collaborators Marcum, Vallee, and Scheele, by sedimentation analysis combined with electron microscopy and gel exclusion chromatography (*J. biol. Chem.* **253,** 2825-2857; 1978). Their preparations of brain microtubule contain species sedimenting at 5.9S (tubulin dimers), 18.6S, and 30.6S when extrapolated to infinite dilution, and will be referred to as 6S, 18S and 30S. By varying the *p*H and the ionic strength they found that the 30S species was the predominant oligomer at low ionic strength and at neutral *p*H (*p*H 6.4–7.4) while the 18S species was found at higher ionic strengths (125–190 mM NaCl) and at higher *p*H values. The construction of a phase diagram suggests that the 18S and 30S species are interconvertible and conditions were found for the conversion of the 18S form to the 30S form and *vice versa.*

The formation of both the 18S and 30S species required the presence of both tubulin and MAPs, with the 18S form being preferentially formed at low MAP : tubulin concentrations. Electron microscopic analysis of preparations containing 23% of the total protein as 18S and the remainder as 6S tubulin shows few rings and it is suggested that the 18S particle is not a ring structure but is formed of 4–6 tubulin dimers with one or more HMW molecules. By contrast the 30S particle is certainly a ring with an outside diameter of 39 nm and a wall thickness of about 4.6 nm. Much of the work described concentrates on the composition and morphology of these 30S rings, because their stability under physiological conditions of *p*H and ionic strength suggests that they may be important *in vivo.*

The dimensions of the 30S ring are at first sight compatible with a single flat ring formed of 6S tubulin dimers arranged end-to-end. However calculations of the sedimentation coefficients of single rings formed of 12 to 15 dimers all yield sedimentation coefficients of 20–25S while double layer rings of 12–15 dimers yield S^0_{20} values of 39–48S. Clearly then single rings formed only of tubulin would sediment too slowly while double rings would sediment too fast. Fractionation of 30S rings by gel exclusion chromatography coupled with gel electrophoresis of the individual fractions indicates that the HMWs are associated with the rings, and no 30S rings are found in tubulin preparations unless supplemented with non-tubulin proteins. The non-tubulin proteins, predominantly HMWs, sediment at 3.4–3.8S so that the simple addition of a few HMW molecules could increase the sedimentation coefficient of the tubulin-HMW complex to the observed 30S. However the HMWs are quite asymmetric when attached to intact microtubules and so would be expected to reduce rather than increase the sedimentation coefficient by increasing the frictional drag. Indeed when the 30S rings were mildly digested with trypsin, which removes 90% of the intact HMW protein without affecting the tubulin or the ultrastructural integrity of the ring, the sedimentation coefficient increased from 30S to 39S. The 30S ring therefore appears to consist of a double stack of rings with a number of integral HMW molecules, with an estimated stoichiometry of one HMW molecule to 5–6 tubulin dimers.

The structure of these 30S rings was further examined by mildly fixing with glutaraldehyde, followed by purification of the rings and electron microscopy by metal shadowing. The rings had a mean height of 18 ± 4 nm, consistent with the hydrodynamic data that they are in fact double rings. The number of tubulin dimers per ring was determined by negative staining, and on the basis of scoring a limited number of micrographs, it was found that there are 13.8 ± 0.1 subunits per circle. On the basis of this ultrastructural and hydrodynamic data the favoured structure for the 30S ring is a complex of 29 tubulin dimers and nine HMW molecules, with the tubulin dimers tilted and distributed along a helix every 7.6 nm.

It was observed that when the 30S rings were separated from the 6S tubulin dimer by ultracentrifugation that the Schieren pattern never returned to base line between the two peaks. This suggested that there is an equilibrium between the 6S and 30S species, and this has been confirmed by an elegant series of experiments of altering the hydrostatic pressure by changing the rotor speed or by partially fitting the cell with mineral oil. For example if the rotor speed is increased after separating the 6S and 30S components at lower speeds, the 30S peak splits with one component continuing to behave as before while the sedimentation rate of the other slows to 6S. The partial specific volume change accompanying this transition was estimated at 9×10^{-4} ml g^{-1}. The positive volume change on polymerisation to the oligomer is very interesting, particularly as it is similar to the value reported by Salmon for the volume change during microtubule polymerisation *in vivo* (Salmon *J. Cell Biol.* **66,** 114-127; 1975).

Finally, Vallee and Borisy have examined the product formed by recombining tubulin with tau. The oligomer was also a ring but sedimented at 20S. The tau–tubulin complex is considered to differ from the 18S oligomer on the grounds that it is seen ultrastructurally as a ring and that its behaviour on dilution differs from that of the 18S particle. Its hydrodynamic properties suggest that it is, unlike the 30S ring, a single ring although the number of tubulin dimers present and the tubulin : tau stoichiometry has not been fully determined.

In some respects these papers by Borisy and his coworkers raise as many questions as they solve. Why do their conditions select for the HMWs rather than the tau stimulatory protein? Are the HMW–microtubules the same as the tau-microtubules? Borisy *et al.* find that only about half of the tubulin was saturated with HMW protein, but suggest that in the intact microtubule this low value may be adequate to organise all of the tubulin. However, what is the effect of loading intact microtubules with addition HMW protein? And finally, what is the relationship of the 18S, 20S and 30S oligomers that Borisy *et al.* have characterised to the other ring structures found by other laboratories? There is still more to be done! □

Corrigendum

In the article 'Complete sequence and secondary structure of a viroid' (*News & Views* **273,** 187; 1978), the sentence "Cutting of the circular structure results in loss of infectivity (Owens *et al. Proc. natn. Acad. Sci. U.S.A.* **14,** 3859; 1977)" should read "Cutting of the circular structure *does not result* in loss of infectivity . . .".

A hundred years ago

THE American Minister for Agriculture has recently stated that in the extensive caverns of Texas enormous masses of guano are deposited. The quantity is estimated at 20,000 tons, and the quality is said to be superior to that of fish guano. Its origin must be looked for in the immense numbers of bats which inhabit these caverns. It is also reported that in the Indian Ocean several guano islands have been discovered, so that the threatened exhaustion of guano deposits need not be feared for some time to come.
From *Nature* **18,** 25 June, 344; 1878.

0028–0836/78/0273—0710$01.00
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articles

Increased atmospheric carbon dioxide and stratospheric ozone

K. S. Groves, S. R. Mattingly & A. F. Tuck
Meteorological Office, Bracknell, Berkshire, UK

A column model in which atmospheric photochemistry and radiative transfer have been coupled together, and with vertical transport represented by eddy exchange coefficients, has been used to calculate the effect of increasing carbon dioxide amounts upon stratospheric ozone. Cooling in the upper stratosphere results in enhanced quantities of ozone.

THERE has been much speculation about the effect of various gases, formed as a result of human activities, on stratospheric ozone. Chronologically, the factors considered have included the emission of nitrogen oxides from the jet engines of supersonic transport aircraft[1], production of nitrogen oxides by nuclear weapon tests[2], production of free chlorine atoms by the stratospheric photodissociation of chlorofluoromethanes[3] and the enhanced atmospheric levels of nitrous oxide following increased use of artificial fertilisers[4]. Official reports published in a number of countries reflect the large research efforts which have been mounted[5-11]. Until recently, when the effect of including methane oxidation and revised rate coefficients has been to indicate an increase in ozone in the lower stratosphere as a result of injections of nitrogen oxides, all the projected effects were such as to cause a reduction of the ozone, resulting in an increased penetration of potentially harmful solar radiation in the wavelength range 290–320 nm to the planetary surface.

In this article we present an assessment of the effect of enhanced amounts of carbon dioxide in the stratosphere (occuring mainly as a result of fossil fuel burning and possibly deforestation) on atmospheric ozone. The attribution of increased atmospheric carbon dioxide to fossil fuel burning was apparently first made by Callendar[12]; reliable measurements of this increase in the last two decades have been made by Keeling and his coworkers[13] and by Bischof[14]. Bolin[15] has reviewed the situation and examined the effect of deforestation. Unlike the other gases considered, CO_2 does not act chemically to perturb ozone. In the upper stratosphere CO_2 emits more infrared radiation to space than it absorbs. Increasing the CO_2 mixing ratio thus cools the upper stratosphere, the region where the majority of the ozone is formed. The ozone forming reaction $O+O_2+M \rightarrow O_3+M$ proceeds rather faster at lower temperatures, and at the same time the various chain reactions which destroy ozone all occur more slowly. It is to be expected therefore that a cooling of the upper stratosphere will increase ozone amounts. This expectation is supported by the correlation of ozone with temperature in dynamically produced stratosperic warmings at the 1 mbar level which has been observed by Barnett *et al*[16].

However, there is a negative feedback mechanism as absorption in the UV and visible by ozone is the basic heating process in the middle and upper stratosphere. Increased ozone results in a larger heating rate because of this absorption and hence in an increase in temperature.

An estimate of the magnitude of these effects can be computed. We have combined a one-dimensional chemical kinetics model, an improvement of that of Tuck[17], with a radiative-convective model similar to that of Manabe and Wetherald[18]. Note that such one-dimensional column models have serious limitations, and should be regarded only as semi-quantitative prognostic tools[19]. However, mainly for reasons of computational economy, one-dimensional models remain the major method available for investigating the chemistry of the atmosphere. Thus this work is a preliminary calculation, which can be used for understanding the effect in an appropriate manner. Ultimately inclusion in models of larger dimensionality, possibly even in a general circulation model, is desirable.

The radiative transfer scheme

The radiation scheme used was similar to that developed by Manabe and Moller[20] and used by Manabe and Wetherald[18], but included full diurnal and seasonal variation of insolation.

Table 1 K_z, vertical eddy transfer coefficients

Altitude (km)	$K_z(m^2 s^{-1})$
47	2.8
45	2.8
43	2.8
41	2.8
39	2.8
37	2.8
35	2.4
33	1.6
31	0.9
29	0.9
27	0.8
25	0.8
23	0.7
21	0.4
19	0.2
17	0.7
15	1.2
13	1.0
11	1.9
9	10.0
7	10.0
5	10.0
3	10.0
1	10.0

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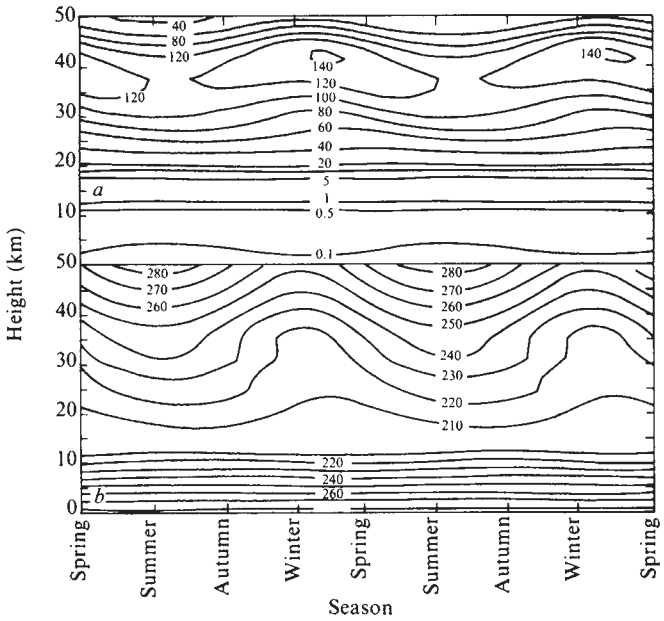


Fig. 1 Experiment 4. Ozone and temperature given by model with $CO_2 = 290 \times 10^{-6}$ VMR during the last 2 yr (that is, at stationary state). *a*, Seasonal variation of O_3 VMR. *b*, Seasonal variation of temperature, K.

The thermal radiative transfer was treated by the use of emissivities to calculate upwards and downwards fluxes and hence cooling rates. The contributions arising from the ozone, water vapour and carbon dioxide bands were considered separately, as was the region of the spectrum where water vapour and carbon dioxide absorption bands overlap. The temperature variation of the emissivities of the various gases was treated explicitly. A single layer of cloud, which was treated as a black body, was incorporated at the 695 mb level of the model; its fractional cover was fixed at 44%.

The solar radiation scheme accounted for the contributions from absorption by water vapour, carbon dioxide and ozone; the effect of Rayleigh scattering was crudely represented by a 7% reduction of the solar constant. The single cloud layer was assumed to reflect 70% of incident solar radiation, and no further account either of this reflected beam or that reflected from the Earth's surface was taken. The cloud was further assumed to scatter diffusely any radiation passing through it. The radiative transfer operated on 14 pressure levels at 1,000, 896, 695, 493, 319, 196, 117, 74, 49, 32, 20, 11, 5, 8 and 1.5 mbar. The cooling rates compared satisfactorily with those from a more complete scheme for a CO_2 VMR (volume mixing ratio) of 350×10^{-6} as described in Hunt and Mattingly[21], who also give details of the Mayer-Goody random band model used to calculate the emissivities employed in our model.

Because our interest focused on the stratosphere, a fixed profile of water vapour volume mixing ratio was used, with a value of 3.5×10^{-6} above 14 km. The surface temperature was fixed at 285 K, and tropospheric temperatures were adjusted to fall linearly between the calculated temperature at 12km and the surface. In practice, this maintained a mean tropospheric lapse rate very close to 6.5K km^{-1}, without the computational complexity of the convective adjustment technique of Manabe and Wetherald[18], who were interested more in tropospheric and surface effects.

The one-dimensional atmospheric chemical kinetics scheme

Our kinetics scheme was similar to a model developed in the Meteorological Office[17] and only the differences from that model are described here. The latitude was set at 34.3 °N as the best compromise for computing ozone perturbations in a hemispherically averaged model. The vertical eddy transfer coefficients, K_z, were the Northern Hemisphere annual average K_z values calculated from the results of the Meteorological Office global, 13-level general circulation model[7], extending from the surface to 2 mbar. The K_z profile is shown in Table 1.

The chemical kinetic scheme (Table 2) was expanded to include a more comprehensive odd hydrogen chemistry and a gross parameterisation of methane oxidation. The solution of these equations has been improved by using a method (S. A. Clough, personal communication) which solves simultaneously for the short-lived species. This allowed longer time steps for the chemistry of 1 and 6 min during the hours of daylight and darkness respectively.

Computational procedure and results

The radiative–convective and chemical kinetics schemes were run as a single, coupled, one-dimensional column model, with the full diurnal and seasonal variation of insolation. Coupling was achieved by updating the temperature profile used in the chemical kinetics scheme with the results from the radiative–convective scheme every 30 (model) min. At the same time, the ozone profile for the radiative calculation was brought up to date by the results from the chemical kinetic calculation. Linear interpolation in altitude coordinate z was used to transfer profiles between the different altitude grids of the two schemes.

Five numerical experiments are reported here. In experiment 1, carbon dioxide was fixed at 325×10^{-6} VMR and initial conditions were those used in the model B experiments described previously[17]. The combined model was run for 10 (model) yr, by which time it had achieved a stationary state, as revealed by comparing the 9th and 10th years day-by-day. Experiments 2, 3, 4 and 5 were all started from the final, stationary state of experiment 1 and were run to new stationary states with fixed carbon dioxide VMR values of 425, 600, 290 and 250×10^{-6} respectively. Each experiment took approximately 100 min of CPU time per simulated year on the Meteorological Office 360/195 computer.

Some of the results are shown in Figs 1–4. The interpretation of time-dependent one-dimensional models has ambiguities which have been discussed previously[17, 19]. Nevertheless, the seasonal phase in the stratosphere above about 30–35 km should be correct because chemical rather than dynamical control occurs above these altitudes in the model. Inspection of

Fig. 2 Experiment 3. Percentage change in ozone and temperature change given by model when CO_2 increased from 290 to 600×10^{-6} VMR. *a*, Seasonal variation of % difference in O_3 VMR, last 2 yr. *b*, Seasonal variation of temperature differences last 2 yr.

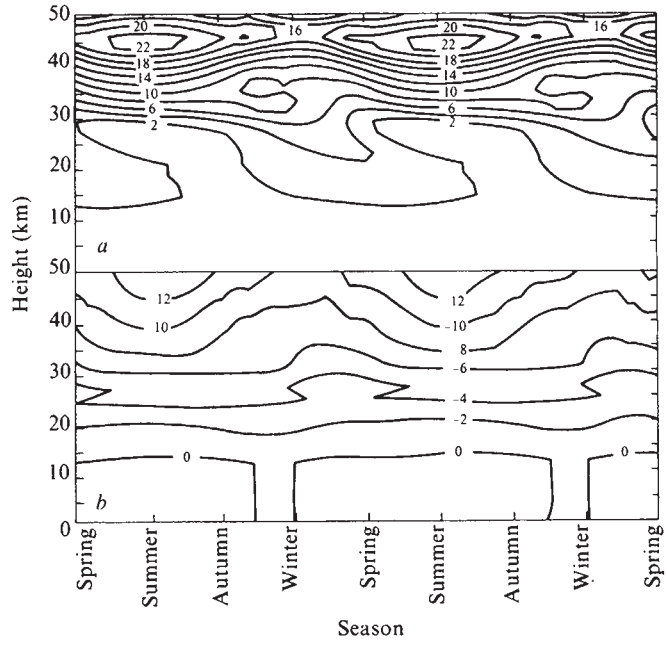


Table 2 Chemical kinetics scheme (rate coefficients in cm mol^{-1} s^{-1} units)

1.	$O_2 + h\upsilon \rightarrow O + O$	Time-dependent photodissociation coefficients calculated every 30 min. Temperature dependence of reactions 2 and 6 (refs. 26 and 27 respectively) included
2.	$O_3 + h\upsilon \rightarrow O(^1D) + O_2$	
3.	$O_3 + h\upsilon \rightarrow O + O_2$	
4.	$NO_2 + h\upsilon \rightarrow NO + O$	
5.	$HNO_3 + h\upsilon \rightarrow OH + NO_2$	
6.	$N_2O + h\upsilon \rightarrow O(^1D) + N_2$	
7.	$NO + h\upsilon \rightarrow N + O$	
8.	$O(^1D) + M \rightarrow O + M$	$2.0 \times 10^{-11} \exp(107/T)$; ref. 11
9.	$O + O_2 + M \rightarrow O_3 + M$	$1.1 \times 10^{-34} \exp(510/T)$; ref. 22
10.	$O + O_3 \rightarrow O_2 + O_2$	$1.9 \times 10^{-11} \exp(-2300/T)$; ref. 22
11.	$O + NO_2 \rightarrow NO + O_2$	9.1×10^{-12}; ref. 11
12.	$NO + O_3 \rightarrow NO_2 + O_2$	$2.3 \times 10^{-12} \exp(-1450/T)$; ref. 23
13.	$O(^1D) + N_2O \rightarrow NO + NO$	5.5×10^{-11}(other channel allowed for); ref. 11
14.	$OH + NO_2 \rightarrow HNO_3$	uv[M]/(u+v[M])*; ref. 29
15.	$OH + O_3 \rightarrow HO_2 + O_2$	$2.3 \times 10^{-12} \exp(-1125/T)$†
16.	$OH + HO_2 \rightarrow H_2O + O_2$	5.1×10^{-11}; ref. 11
17.	$O(^1D) + H_2O \rightarrow OH + OH$	2.3×10^{-10}; ref. 11
18.	$O + HO_2 \rightarrow OH + O_2$	3.5×10^{-11}; ref. 11
19.	$N + NO \rightarrow N_2 + O$	$8.2 \times 10^{-11} \exp(-410/T)$; ref. 11
20.	$N + O_3 \rightarrow NO + O_2$	$5.0 \times 10^{-12} \exp(-650/T)$; ref. 22
21.	$NO + HO_2 \rightarrow NO_2 + OH$	1.6×10^{-12}; ref. 11
22.	$O + OH \rightarrow O_2 + H$	$1.0 \times 10^{-10} \exp(-250/T)$; ref. 25
23.	$HO_2 + O_3 \rightarrow OH + O_2 + O_2$	$1.0 \times 10^{-13} \exp(-1250/T)$; ref. 22
24.	$H + O_2 + M \rightarrow HO_2 + M$	$2.1 \times 10^{-32} \exp(290/T)$; ref. 11
25.	$H + O_3 \rightarrow OH + O_2$	$1.2 \times 10^{-10} \exp(-560/T)$; ref. 11
26.	$OH + CH_4 \rightarrow CH_3 + H_2O$	$2.4 \times 10^{-12} \exp(-1710/T)$; ref. 11
27.	$O(^1D) + CH_4 \rightarrow CH_3 + OH$	1.3×10^{-10}; ref. 11
28.	$CH_3 \rightarrow 2HO_2$	Parameterisation; CH_3 radicals have lifetime of 100 s.

* $u = (68.0 - 0.22T)10^{-12}$, $v = 3.7 \times 10^{-32} \exp(1093/T)$
† R. A. Cox, personal communication.

Fig. 2, which shows the ozone and temperature changes caused by increasing the CO_2 in the model from 290 to 600×10^{-6} VMR reveals the direct association of cooling with ozone increases. In summer, the increase in ozone in the 25–30 km region is less than in winter; this is because the increase in ozone at higher levels results in relatively more UV absorption aloft and hence relatively less UV radiation penetrates to 25–30 km. This effect is therefore the reverse of the well known self-healing effect associated with calculated ozone reductions as a result, for example, of photodissociation of chlorofluoromethane molecules in the upper stratosphere.

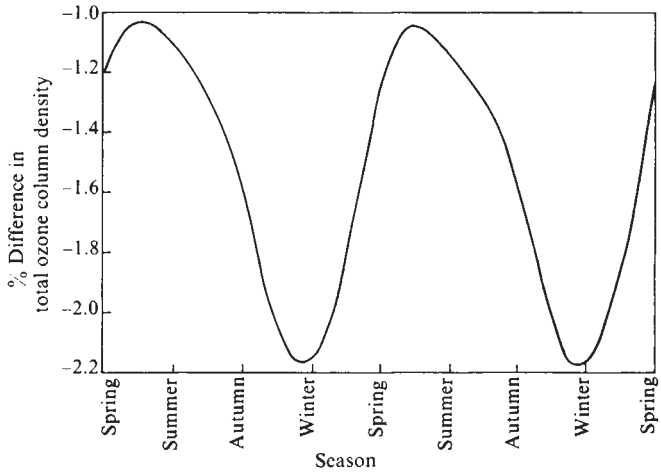
In terms of ozone column density, the changes show considerable seasonal dependence. Figure 3 shows the percentage changes of ozone column density in experiment 5, in which carbon dioxide was fixed at 250×10^{-6} VMR compared with those of experiment 4 with 290×10^{-6} VMR. The decreases in ozone column density in experiment 5 relative to experiment 4 are approximately 2.2% in winter, and 1% in summer. The seasonal dependence of the percentage change in ozone column density has its maximum in winter although Fig. 2 shows larger changes in ozone VMR in summer. The number density of ozone, from which column density is calculated, has a maximum in the model at about 25 km, where percentage changes in ozone VMR are greater in winter. It is the contributions to the total column density from this region which outweigh the phase of the larger changes at higher altitudes.

Using the data of Bolin[15] and of Keeling and his coworkers[13,30] it is possible to assign approximate dates to the carbon dioxide mixing ratios used in the model experiments. Bolin[15] estimated that the mixing ratio may have been as low as 275×10^{-6} at the beginning of the nineteenth century, while Bacastow and Keeling[30] estimated the pre-industrial carbon dioxide content of the atmosphere as 290×10^{-6} VMR and that this would double by the year 2030. We assume a value of 275×10^{-6} for the year 1800, establishing the column density of ozone by interpolation between experiments 4 and 5. The carbon dioxide VMR values of 290, 325, 425 and 600×10^{-6} are identified respectively with the years 1925, 1972, 2020 and 2030. Table 3 shows the percentage change in ozone column density as a function of carbon dioxide VMR, relative to 275×10^{-6} (that is, 1800). The simplifying assumptions in our radiative–convective scheme are not valid much beyond a factor of two change from 350×10^{-6} carbon dioxide VMR, and also neglect dynamical effects in the stratosphere, so we cannot compute the effects of the rapid exponential growth projected by Bacastow and Keeling[30]; according to their Fig. 8, between 2030 and 2050, the atmospheric carbon dioxide amount would more than double again.

Historical measurements of atmospheric CO_2 and O_3

The only two trace gases for which there is some historic information from which a time-series may be constructed are ozone and carbon dioxide. Figure 4 shows the deviations from the monthly means of total ozone column densities derived

Fig. 3 Experiment 5. Percentage change in ozone column density given by model when CO_2 reduced from 290 to 250×10^{-6}, last 2 yr.



from a combination of the Oxford and Arosa (Switzerland) Dobson spectrophotometer observations, which have been made since 1925. These data were displayed in this form, separately, in Goldsmith *et al*[31]. The deviations from the monthly means are shown as annual averages in Fig. 4. The dashed line is the mean linear slope, which has a value of 0.12% per yr (approximately 0.4 Dobson units), with a standard deviation of 0.02%. According to this treatment of the data, the ozone column density has increased by about 6% in the period 1925–72, for the mean of the two stations. This compares with an increase of 0.5% in the ozone column density calculated by the model as a result of increasing CO_2. However, the nature of the ozone measurement technique[32], and the changes which have been made since 1925, mean that these ozone data are not as reliable in establishing long-term trends as might be desired, especially when considering small, long-term percentage changes.

Carbon dioxide measurements from the early part of the century also show a scatter which makes it difficult to argue quantitatively. In view of the immense, interactive complexity of the biospheric–oceanographic–atmospheric system, and the limitations of both data and model, it is perhaps encouraging that the actual measurements of ozone show a trend in the same direction as that deduced from our model. Parry[33] has reported a 4% increase in Northern Hemisphere ozone between 1958 and 1975, using up to 39 Dobson station results; this result, although in the same direction as our calculation, is larger by an order of magnitude. There are, of course, many meteorological factors, such as tropopause height, which may also affect long-term ozone column densities.

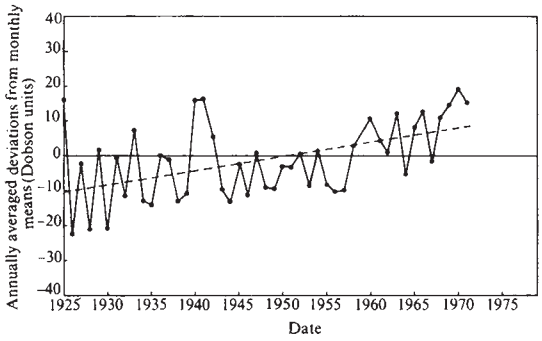


Fig. 4 Annual means of monthly deviations from mean, Oxford plus Arosa Dobson stations, 1925–72. Straight line represents mean slope of 0.12% per yr; s.d. = 0.02%.

Discussion and conclusions

It is of interest to compare the ozone increases calculated for the year 2030 with those computed in a recent report[9], since these were also obtained by one-dimensional modelling. The decrease in ozone column density attributable to chlorofluoremethanes in 2030, assuming continued steady release at 1973 rates, was about 4%, with a steady-state reduction of 6–7.5% attained a few decades later. The corresponding figure caused in 2030 by rises in carbon dioxide amounts to an increase of 4.5% in summer, and 6.5% in winter. According to these calculations the year 1972 itself has approximately a 2% increase in winter ozone column density compared to an atmosphere with 275×10^{-6} VMR of carbon dioxide, with a 1% summer ozone increase, both figures referring to a hemispheric mean.

The ozone reductions computed to result from chlorofluoromethanes were approximately doubled[11] by changing the rate coefficient of reaction (21) in Table 2 to 8×10^{-12}. Nevertheless, the time scale for carbon dioxide doubling by the middle of next century may still mean that there is a substantial compensating ozone production. It cannot be assumed that ozone perturbing effects are linearly additive[17], and the construction of a chlorofluorocarbon stratospheric chemical kinetics model interactive with a radiative transfer scheme capable of handling much larger carbon dioxide increases than 100% is necessary; the infrared absorption by chlorofluoromethanes in the window region from 8–13 μm wavelength region would also need to be included.

Table 3 O_3 column density perturbations and CO_2 volume mixing ratio

CO_2 (VMR $\times 10^6$)	% Change in O_3 col density (annual mean)	Year
250	−0.9	—
275	0.0	1800
290	0.8	1925
325	1.3	1972
425	2.3	2020
600	5.5	2030

The general circulation model study of Manabe and Wetherald[34] indicated an increase in the intensity of the hydrological cycle of ~7% as a result of increasing carbon dioxide to 600×10^{-6} VMR. Since the OH and HO_2 molecules have key roles in controlling ozone above 40 km in stratospheric models, this would be very important if increased carbon dioxide were to result in a change of tropopause temperature, because this might be expected to alter the supply of water vapour to the stratosphere through the equatorial 'cold trap'. Any change in methane amounts as a result of changes in biospheric or human activity would also affect the supply of OH and HO_2 in the upper stratosphere.

The model calculations performed here, in common with other one-dimensional studies, also take no account of the sensitivity of the atmospheric circulation to changes in the radiative field in the lower stratosphere; Harwood[35] has noted that this could cause changes of tens of Dobson units in the total ozone column density. Similarly, the effects of changed temperature in our model would cause changes in static stability. According to the calculations of Bates[36], it is possible that this may affect the tropospheric circulation by altering the upwards propagation of energy in ultra-long planetary scale waves in mid-latitudes. Our calculations cannot encompass any such complexities, because of the crude, fixed parameterisation of atmospheric dynamics which is an adjunct of one-dimensional modelling. Nevertheless, the computations indicate significant increases in upper stratospheric ozone as a result of increases in CO_2. They also suggest that any future increases in the total ozone column density due to increased CO_2 loading of the atmosphere are not negligible when compared with present estimates of future decreases of total ozone caused by increased chlorofluoromethane levels in the atmosphere, which are also based on one-dimensional models subject to the same limitations. However, as perturbing effects on stratospheric ozone amounts are known not to be linearly additive in model calculations, there is a need when estimating possible future perturbations to consider them all, including CO_2, in a single model computation.

After completion of this study a recent publication by Luther *et al*[37]. became available. They report one numerical experiment similar to our experiment 3, in which a 2.9% increase in ozone column density was computed as a result of doubling CO_2 to 600×10^{-6} VMR. This is about half our result; their temperature changes are similar to ours. The main difference in the computed ozone perturbations seems to arise below 30 km. This emphasises the importance of the dynamics in transferring ozone to the lower stratosphere where it is well conserved. Of the three fundamental processes determining stratospheric ozone distributions–photochemistry, radiation and dynamics–the last of these is much the least well represented in our model; it is also non-iteractive.

Note added in proof: The difference in ozone column density perturbation compared to ref. 37 probably lies in our use of the data of Johnston and Selwyn in reaction (6); see also ref. 38.

Received 7 March; accepted 5 May 1978.

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Transitional field configurations and geomagnetic reversal

K. A. Hoffman*

Department of Geology and Geophysics, University of Minnesota, Minneapolis, Minnesota 55455

M. Fuller

Department of Geological Sciences, University of California, Santa Barbara, California 93106

Records of various geomagnetic reversals which occurred over the past 25 Myr suggest that transitional field configurations are largely controlled by low-order zonal components. Given sufficient data further clarification is possible as octupole and quadrupole dominated fields are palaeomagnetically distinguishable. Moreover, because each geometry may reflect a reversal process which starts in a particular region of the core, configurational characteristics of the geomagnetic dynamo during polarity transitions are, in principle, identifiable.

BEHAVIOUR of the geomagnetic field during transitions has been revealed, sometimes in considerable detail, through palaeomagnetic records which happen to span a reversal[1,2]. Each record, however, only displays behaviour experienced at one particular site. Nevertheless, analysis of such records, through the determined transitional path of the virtual geomagnetic pole (VGP), have shown certain characteristics to be common to many transitions. It is of particular interest that most detailed transitional VGP paths are largely constrained in longitude during the excursion from one polarity to the other[2,3]. Two such paths from widely separated sites which recorded the Matuyama–Brunhes reverse-to-normal (R→N) transition were found to be widely dissimilar, thus providing clear evidence that the ambient field during this reversal was predominantly non-dipolar[4]. Further clarification of the geometry of transitional fields has been made recently with the recognition of a dependence of transitional VGP path behaviour on site location[5]. Moreover, this site-control was shown to be consistent with a transitional field which is predominantly zonal, a field geometry suggestive of a hydromagnetic reversal process which starts at certain latitudes in the core[5].

Both octupole[5] and quadrupole[2] dominated transitional field geometries have been hypothesised. We show here that these field configurations and, hence, primary characteristics of the reversing dynamo process from which each may arise, are palaeomagnetically distinguishable. We also indicate more specifically which cases are possible, although the lack of available data precludes any firm conclusion.

Axisymmetric models

Let us consider separately two axisymmetric, or zonal, non-dipole field configurations and compare the effect each would have on transitional VGP behaviour if assumed to be intermediate states of the field during reversals. One such configuration—type A—is predominantly octupolar and, hence, antisymmetric about the Equator. A type A transitional field geometry would be a consequence of a hydromagnetic reversal process which starts at either low or high latitudes, and subsequently floods to the remaining regions of the core so as to complete the reversal. Such a reversal process has been suggested for the Earth[6–8] as well as for the Sun[9].

* Permanent address: Physics Department, California Polytechnic State University, San Luis Obispo, California 93407.

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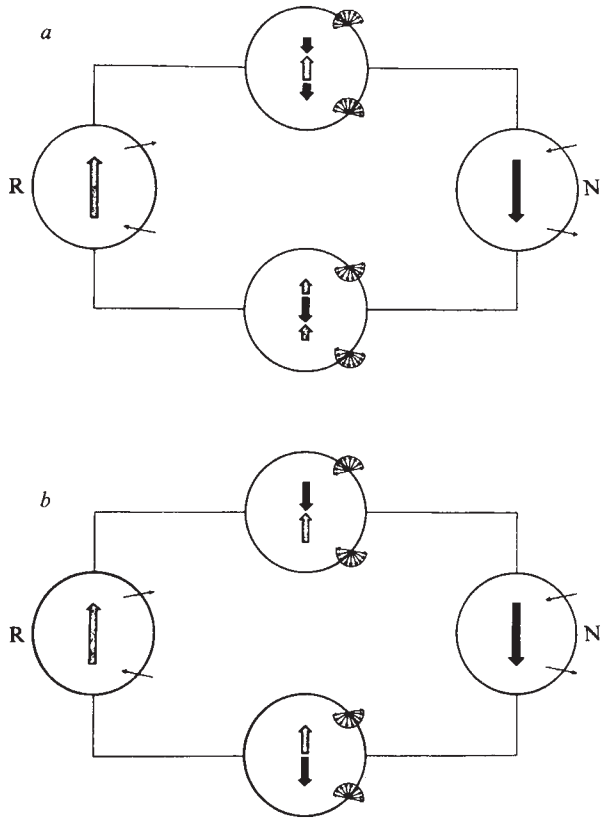


Fig. 1 Reversal sequences corresponding to type A (*a*) and type S (*b*) transitional fields. R→N and N→R transitions run from left to right and from right to left, respectively. For each reversal sequence the succession of intermediate field directions experienced at mid-latitude sites in the Northern and Southern Hemispheres is displayed.

The other axisymmetric transitional field considered here—type S—is predominantly quadrupolar and, hence, symmetrical about the Equator. A type S transitional field geometry would be expected from a flooding process where one hemisphere of the core reverses before the other[2].

These two types of possible transitional field geometries are readily distinguishable palaeomagnetically if reversal records from both northern latitudes and southern latitudes are available. For example, if during a reversal the transitional field is of type A (Fig. 1*a*), then the field vector at any site in one hemisphere will at some time during the reversal rotate through a position having +90° inclination, at which point the VGP and the site are coincident. Sites in the other hemisphere, on the other hand, will experience a field direction which rotates through a position having −90° inclination, at which point the VGP resides at the point antipodal to the site. Hence, type A transitional field behaviour requires the observed path of the VGP to run along the site meridian for all sites in one hemisphere and, similarly, a path which is confined to the meridian antipodal to the site for all sites in the other hemisphere[5]. More realistically, when non-axisymmetric field components are also invoked, all VGP paths will deviate from either of these particular meridians so as to be found preferentially on either the hemisphere centred about the site meridian, the near side, or the hemisphere centred about the meridian antipodal to the site, the far side. The side on which a given path resides depends on the sense of the transition, the location of the site and whether low or high latitudes of the core reverse first.

In contrast, for the case of a type S transitional field (Fig. 1*b*), all sites, regardless of hemisphere will experience a field rotation either through +90° or −90° inclination, depending on the sense of the transition and whether the Southern Hemisphere or Northern Hemisphere of the core reverses first. Hence, given the presence of some non-axisymmetric field components as well, all VGP paths corresponding to a particular transition will preferentially reside on the near side or on the far side, independent of the site location.

Each of the sequences of field configurations in Fig. 1 corresponds to a reversal process which starts in a particular region of the core. Note that R→N transitions run from left to right and N→R transitions from right to left. For the two cases involving a type A field geometry (Fig. 1*a*), for R→N transitions the upper sequence corresponds to initial reversal at high latitudes and the lower sequence to a reversal beginning at low latitudes; for N→R transitions the opposite applies. Similarly, for type S field geometry (Fig. 1*b*), the two sequences correspond to reversals which start in the Northern or Southern Hemisphere.

By considering type A and type S models separately, one may construct eight complete reversal cycles (Fig. 2) by matching each R→N sequence with either N→R sequence from the same diagram in Fig. 1. The reversal regimes on the left of Fig. 2 correspond to hydromagnetic processes which are independent of the sense of the transition (for example, low latitudes in the core reverse first for all transitions). Those regimes on the right of Fig. 2 reflect a process which depends on the transition

Table 1 Reverse-to-normal transition data

Site description	Coordinates	Age	Rock type	N*	$\langle\Delta\phi\rangle$†	Refs
Redhill, Czechoslovakia	49° N, 16° E	Matuyama–Brunhes	Sediments	16	61.8(9.4)	10
Boso Peninsula, Japan	35.3° N, 140.2° E	Matuyama–Brunhes	Sediments	11	−19.4(8.5)	11
North-Central Pacific	38.5° N, 190.0° E	Matuyama–Brunhes	Sediments	4	34.0(26.8)	12
Mt Rainier, Washington	46.8° N, 238.2° E	18.0±0.5 Myr	Intrusion	5	−49.1(17.3)	2, 13
Mt Hood, Oregon	45.5° N, 238.3° E	8.2±0.5 Myr	Intrusion	15	−55.4(5.6)	2, 14
Steens Mt, Oregon	42.6° N, 240.5° E	15 Myr‡	Lavas	6	−21.0(22.2)	15
Santa Rosa Range, Nevada	41.9° N, 242.3° E	15.1±0.3 Myr‡	Lavas	8	38.1(25.1)	16
Lake Tecopa, California	35.8° N, 242.7° E	Matuyama–Brunhes	Sediments	9	99.8(5.7)	4
East-Equatorial Pacific	8° N, 269–273° E	Matuyama–Brunhes	Sediments	8§	51.6(10.3)	17
Western Iceland	64.3° N, 338.6° E	Pliocene	Lavas	17	125.8(6.9)	18
Eastern Iceland	65° N, 346° E	Miocene	Lavas	9	−100.3(13.3)	1
		Miocene	Lavas	5	−96.2(43.2)	1
		Miocene	Lavas	6	−66.6(15.7)	1
		Miocene	Lavas	5	−134.7(30.5)	1
Daroca, Spain	41.1° N, 358.7° E	Miocene	Sediments	3	−27.0(20.1)	19

Sites are listed in order of increasing east longitude. Data sets having less than three transitional poles were not considered.
* No. of transitional VGPs.
† Mean angular longitudinal distance of transitional VGPs from site meridian (+) east of site, (−) west of site; s.e.m. are given in parentheses.
‡ Thought to be the same reversal.
§ Total from three separate cores.

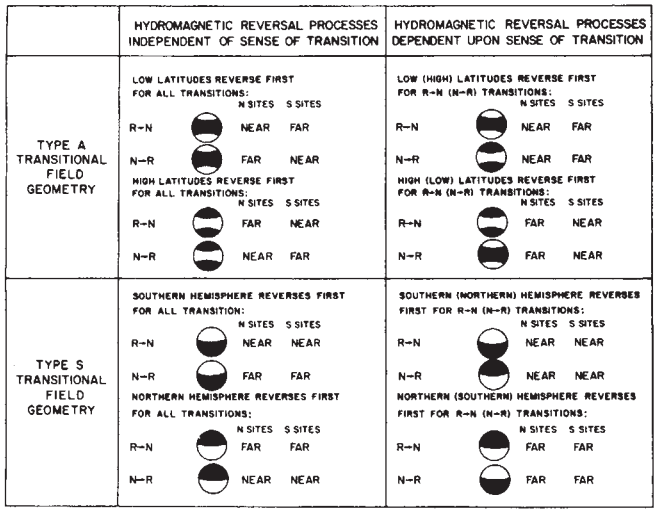
sense (for example, low latitudes reverse first for R→N transitions and high latitudes reverse first for N→R transitions). Moreover, each regime, corresponding to a particular pair of transitional field geometries, has a unique set of predicted VGP paths when all transition sense/site location combinations are considered. Hence, configurational characteristics ,of the reversing geomagnetic dynamo are palaeomagnetically distinguishable provided that a sufficient number of both R→N and N→R transition records are available from sites in both Northern and Southern Hemispheres.

Discussion

The question therefore is: do we have enough palaeomagnetic data to make conclusive statements on the geometry of transitional fields and, hence, the nature of the reversing geomagnetic dynamo? The answer is, unfortunately, no; however, available data do provide clear evidence that at least during R→N polarity transitions the field passes through a configuration consistent with some of the reversal cycles shown in Fig. 2.

Focusing our attention on R→N reversals we enlarge on the transitional data presented elsewhere[5] by including several more records. Table 1 lists information regarding several Miocene and younger R→N transition records from sites in the Northern Hemisphere. Included for each data set are the number of transitional VGPs (those which lie between 45° N and 45° S latitude) as well as the mean angular longitudinal distance that these VGPs are found from the site. Also given is the standard error of the mean to indicate the dispersion of transitional pole positions; however, the values should be considered with caution as some data sets reflect a previous smoothing of the raw records but others do not. Figure 3*a* is a polar stereographic projection displaying the location of all these transitional VGPs. Although the density of VGPs is not homogeneous in longitude, preferred bands[3,20] are not discernible. The same data are displayed in Fig. 3*b*, but here the data sets were first individually rotated to align all sites along a common meridian. As suggested elsewhere[5] the transitional VGP data are no longer randomly distributed, but are found more often in the hemisphere centred about this common site meridian, that is, on the near side. Moreover, this behavior becomes dramatically apparent when transitional data from Iceland are excluded (Fig. 3*c*). These VGPs, representing the

Fig. 2 Possible cyclic reversal processes which involve type A and type S transitional field geometries. For each regime the set of VGP path characteristics is indicated for the four possible site location/transition sense combinations. 'N' and 'S' refer to the Northern and Southern Hemispheres, respectively. 'Near' and 'far' indicate VGP path location on the hemisphere cnred about the site meridian and on the hemisphere centred about the meridian antipodal to the site, respectively. The darkened portion of each figure indicates the region(s) in the core where the reversal is initiated.



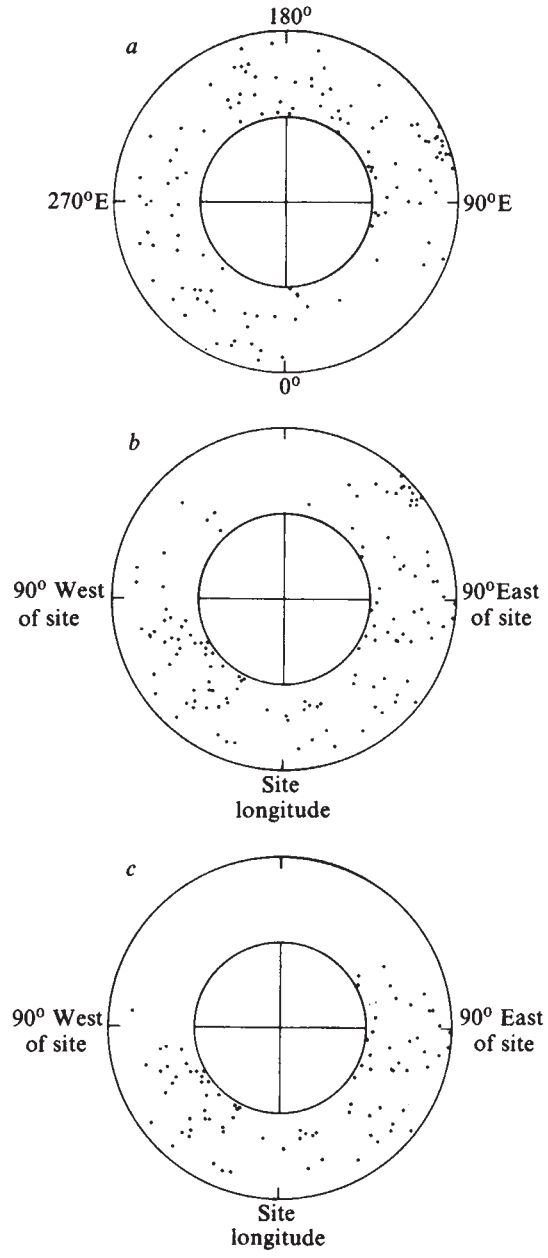


Fig. 3 Polar stereographic project of transitional VGPs from data sets listed in Table 1 and plotted *a*, against geographic longitude; and *b*, with respect to a common site meridian; *c*, is the same as *b*, without transition data from Iceland. Northern Hemisphere VGPs are not distinguished from those occurring in the Southern Hemisphere.

only sets of unsmoothed data from sequences of lava flows, are in some cases associated with a high degree of dispersion (see Table 1). However, it is unclear why other transitions, most notably the unusual '$R_3 \rightarrow N_3$' path from Western Iceland[18,21], display behaviour having little in common with those from all other sites listed in Table 1. One possibility is that during reversals the effect of high order non-dipole field components is more pronounced at sites having such a high latitude. In any case, Fig. 3*c* displays R→N transitional VGPs from all other (10) sites in the Northern Hemisphere and not one of the 85 plotted data points is found beyond 125° to the east or 95° to the west of the common site meridian. Note that these sites have latitudes ranging from 8° N to 49° N and are distributed reasonably well in longitude (Table 1). This striking behaviour strongly suggests that, at least for sites at low or mid latitudes in the Northern Hemisphere, a low-order axisymmetric component of the geomagnetic field predominates sufficiently to constrain the path of the VGP during reversals.

Unfortunately, there are not enough N→R transition records from Northern Hemisphere sites, or transitional data of either sense recorded at southern latitudes for a meaningful comparison with Fig. 3*b* and *c* (see refs 1–3). Hence, we cannot firmly distinguish which of the eight reversal schemes depicted in Fig. 2 may actually occur. However, we may reduce the number of possibilities to four, as half of the regimes listed in Fig. 2 predict far-sided VGP behaviour for R→N transitions recorded at sites in the Northern Hemisphere, in conflict with the observed data (Fig. 3*b*,*c*). Further clarification on the geometries of transitional fields and, hence, the hydromagnetic reversal process, can only be made using additional reversal records. Nevertheless, we may speculate, given the data available. Although few detailed N→R transition records are available, those recorded in the Northern Hemisphere tend to display far-sided VGP behaviour[5]. Confirmation of such a trend would rule out two additional schemes in Fig. 2 leaving either, (1) type A behaviour consistent with a reversal process which occurs first at low latitudes in the core for all transitions, or (2) type S behaviour consistent with a reversal process which occurs first in the Southern Hemisphere of the core for all transitions. Both possibilities correspond to a dynamo process which is independent of the sense of the transition, that is, the sign of the field. Such a conclusion seems to be sensible in the light of theoretical considerations[22]. Finally, distinguishing between the above possibilities requires detailed reversal records from sites in the Southern Hemisphere which are at present almost completely absent.

We thank W. K. Freed for his unpublished data and S. Lund for helpful discussions.

Received 3 March; accepted 19 April 1978.

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Alterations in the surface properties of cells responsive to nerve growth factor

D. Schubert, M. LaCorbiere, C. Whitlock* & W. Stallcup

The Salk Institute, P.O. Box 1809, San Diego, California 92112 and * Department of Medical Microbiology, University of California, Irvine, California

An initial effect of nerve growth factor on a responsive nerve cell line is to increase intracellular cyclic AMP, which in turn leads to a mobilisation of calcium ions. These events are correlated with structural changes in the plasma membrane, increased cell–substratum adhesion, and neurite outgrowth.

NERVE GROWTH FACTOR (NGF) has a major role in the development and maintenance of sympathetic neurones (for review see ref. 1). Although much is known about the physiological consequences of the NGF–cell interaction, little information has been obtained about the molecular mechanisms which generate these effects. One of the best documented *in vitro* effects of NGF which is analogous to that observed *in vivo* is the induction of neurite outgrowth. This morphological change occurs in explant cultures[1] and dissociated cell cultures[2] of sympathetic ganglia. Recently, a clonal cell line was isolated[3] which shares many properties with dissociated sympathetic ganglion cells in cultures. This clonal cell line, PC12, responds to NGF with neurite outgrowth[3], synthesises and secretes catecholamines and acetylcholine[3–6], and forms cholinergic synapses with a clonal skeletal muscle cell line[4], all of which are properties shared with primary cultures of dissociated sympathetic ganglion neurones[7,8]. Thus, it is likely that mechanisms which underlie the response of PC12 cells to NGF are similar to those used by the sympathetic neurone.

It has been argued from the study of several clonal nerve cell lines that conditions which lead to increased cell–substratum adhesion favour neurite outgrowth[9]. If this mechanism were generally used, then NGF may induce neurite outgrowth in sympathetic ganglion cells by altering the cell surface structure to increase cell–substratum adhesion. In an initial study of this possibility, it was demonstrated that NGF increased cell–substratum and cell–cell adhesion in PC12 cells, and that intracellular cyclic AMP may mediate this process[10]. The experiments described here extend these studies to show that calcium mobilisation may ultimately be responsible for increasing cellular adhesion, and that these events are correlated with increased lectin agglutinability.

Relationship between cell–substratum adhesion and neurite extension

If increased cell–substratum adhesion mediates neurite extension, then there should be a direct correspondence between the concentrations of NGF required to increase adhesion and to induce neurite extension. To assay for cell–substratum adhesion, low density PC12 cultures were labelled overnight with ^{3}H-leucine, washed with HEPES-buffered medium containing 10% fetal calf serum, and plated into 35-mm tissue culture dishes containing 2 ml of the same medium and the test compound[10]. The number of attached cells was then determined as a function of time, and the data plotted as the percentage of the input cells which adhered. Three conditions were tested: NGF and dibutyryl cyclic AMP, which have previously been shown to increase cell–substratum adhesion[10], and 50 mM exogenous potassium, which is required for the survival of nerves in some primary cultures.[11]. Figure 1 shows that all three reagents enhanced cellular adhesion. In addition, there

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was excellent correspondence between the concentrations of potassium and NGF required for adhesion and for neurite extension (Fig. 2); dibutyryl cyclic AMP was functional in both assays only at concentrations greater than 2×10^{-4} M. Both the initial adhesion rate and neurite extension saturated at 10 ng ml^{-1} of β-NGF (4×10^{-10} M) and 40 mM potassium.

The role of cyclic AMP and calcium in neurite extension

As NGF stimulates intracellular cyclic AMP accumulation[10], and both NGF and dibutyryl cyclic AMP stimulate neurite extension and cell–substratum adhesion[10], cyclic AMP may play the part of 'second message' for NGF. If this were true, then other conditions which stimulate intracellular cyclic AMP accumulation should increase both cellular adhesion and neurite extension. Thus, phosphodiesterase inhibitors should potentiate the effect of NGF. The data presented in the upper part of Table 1 show that cholera toxin, which increases intracellular cyclic AMP in mammalian cell lines[12], enhanced both cell–substratum adhesion and neurite extension. Although ineffective when used alone, theophylline potentiated the effect of both NGF and cyclic AMP on the rate of adhesion. Finally, vinblastine enhanced the rate of cell–substratum adhesion to the greatest extent of all reagents or conditions tested, but was cytotoxic to the cells over periods long enough to assay neurite extension (Table 1). The adhesive response caused by vinblastine could be mediated through an increase in intracellular cyclic AMP but possibly by some other mechanism. The data presented in Table 2 show that 1×10^{-5} M vinblastine elevated cyclic AMP levels in PC12 cells to a much greater extent than NGF. Thus, the above data indicate that cyclic AMP is involved in the NGF response of the cells,

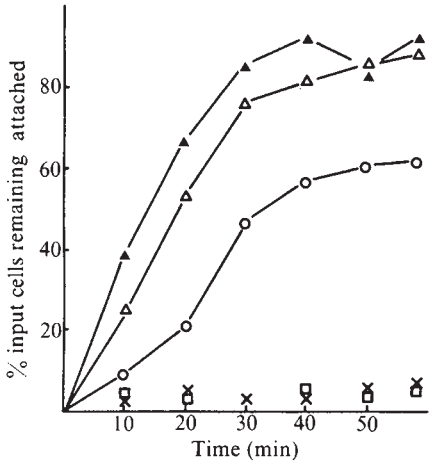


Fig. 1 Effect of NGF, cyclic AMP and 50 mM K^+ on cell–substratum adhesion. Exponentially growing cells at a density of 1×10^6 cells per 100-mm tissue culture dish were labelled overnight with ^{3}H-leucine in complete growth medium (see ref. 10 for details). The cells were washed and plated at 3×10^4 cells per 35-mm tissue culture dish (Falcon, lot no. 63221408) into 2 ml of HEPES-buffered modified Eagle's medium[10] at 22°C containing 10% fetal calf serum and the indicated reagents. The initial rate of adhesion was determined at intervals by manually swirling the dish six times, aspirating the medium, dissolving the attached cells in detergent, and counting the isotope in a scintillation counter[10]. Variation between duplicate samples was less than 10%. The data are plotted as the % of the input cells remaining attached to the surface as a function of time. The number of cells remaining if the medium was removed immediately was less than 1.5% of the input. All experiments were repeated at least twice with similar results. ×, Control, no additive; ○, 1×10^{-3} M cyclic AMP plus 1×10^{-3} M theophylline; △, 10 ng ml^{-1} β-NGF; ▲, 50 mM potassium, substituted for sodium; □, 10 ng ml^{-1} β-NGF preincubated for 30 min with a 1:40 dilution of heat-inactivated anti-NGF serum.

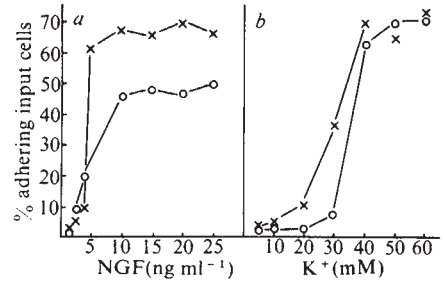


Fig. 2 Dose–response relationship for adhesion and neurite extension. β-NGF was added to low-density exponentially dividing PC12 cultures at the concentrations indicated. Culture medium was also removed from cells and replaced with identical medium in which NaCl was substituted with increasing concentrations of KCl. Three days later the % of cells with neurites greater than the cell diameter was scored. Adhesion assays were carried out as described in Fig. 1, and the data presented as the % of the input cells which had adhered to the culture dish at 30 min. *a*, Various concentrations of NGF. ×, Neurite extension; ○, cell–substratum adhesion. *b*, Potassium concentrations. ×, neurite extension; ○, adhesion.

but is it the final effector in the NGF-triggered chain of events leading to neurite extension? A further investigation of the effect of elevated potassium on adhesion and neurite extension suggests that it is not.

The simplest alternatives which could explain the positive effect of 50 mM potassium on adhesion and neurite outgrowth are that it functions by elevating intracellular cyclic AMP or by depolarising the cells. As experiments measuring the effect of 50 mM potassium on intracellular cyclic AMP repeatedly failed to show any stimulation (Table 2), other conditions which should decrease the resting potential of PC12 cells were examined. Table 1 shows that both ouabain and veratridine stimulate cell–substratum adhesion; veratridine also induces neurite outgrowth. In addition, tetrodotoxin blocks the effect of veratridine on adhesion and neurite outgrowth, and scorpion (*Tityus serrulatus*) toxin and/or ouabain potentiate the effect of veratridine on adhesion. The effectiveness of these reagents in stimulating cell–substratum adhesion follows that found in PC12 for stimulating $^{22}Na^+$ influx and thus membrane depolarisation[13].

The above data suggest that membrane depolarisation is sufficient to cause increased adhesion and neurite extension. However, if it were a necessary factor, then NGF and cyclic AMP should cause membrane depolarisation. There is no precedent for such an effect of these compounds on membrane potential, and an analysis of sodium flux in their presence shows no stimulation (W.S., unpublished observation). Membrane depolarisation does, however, activate a membrane voltage-dependent calcium channel in PC12 cells[13] which leads to an influx of calcium ions. Data in the right hand column of Table 1 show that all conditions which depolarise the cell result in a net influx of calcium. When the potassium-induced calcium influx is blocked by cobalt ions, cell–substratum adhesion is also eliminated. Carbachol also stimulates adhesion and calcium influx; these responses are blocked by the nicotinic antagonist curare but not by atropine. If an alteration in free calcium activity is responsible for the changes in the plasma membrane which lead to increased cell–substratum adhesion, then NGF and cyclic AMP should mobilise calcium, and an ionophore which stimulates calcium influx should enhance adhesion. Table 1 shows that the calcium ionophore A23187 stimulates cell–substratum adhesion at concentrations between 1 and 10 μM. Figure 3 shows that NGF, cyclic AMP and dibutyryl cyclic AMP stimulate the efflux of calcium from cells preloaded with $^{45}Ca^{2+}$; these reagents do not detectably stimulate $^{45}Ca^{2+}$ uptake. Vinblastine also stimulates calcium efflux, but not calcium uptake (see legend of Fig. 3). The stimulatory

Table 1 Effect of several reagents on cellular adhesion, neurite extension and calcium flux

Condition	Concentration	Adhesion	Neurite extension	Calcium flux
Control	—	3	−	1.0±0.1
β-NGF	20 ng ml^{-1}	48	+	See Fig. 3
Cyclic AMP	1×10^{-3} M	23	−	
Theophylline	1×10^{-3} M	4	−	
β-NGF+theophylline	as above	65	+	
Cyclic AMP+theophylline	as above	41	+	See Fig. 3
Cholera toxin	1 μg ml^{-1}	55*	+	
Vinblastine	1×10^{-5} M	86	†	See Fig. 3
Cobalt	2×10^{-3} M	2	‡	1.0±0.1
Potassium	5×10^{-2} M	63	+	13.5±0.5
Potassium+cobalt	as above	4	‡	1.1±0.1
Ouabain	5×10^{-3} M	25	‡	1.5±0.1
Veratridine	2×10^{-4} M	30	+	1.4±0.1
Scorpion toxin	100 μg ml^{-1}	5	−	1.0±0.1
Tetrodotoxin	1×10^{-6} M	5	−	1.0±0.1
Veratridine+tetrodotoxin	as above	4	−	1.0±0.1
Veratridine+scorpion toxin	as above	60	+	1.9±0.1
Ouabain+veratridine+scorpion toxin	as above	68	‡	2.4±0.2
Carbachol	5×10^{-4} M	18	−	2.3±0.2
Curare	5×10^{-4} M	4	−	1.0±0.1
Atropine	5×10^{-6} M	5	−	1.0±0.1
Carbachol+curare	as above	5	−	1.0±0.1
Carbachol+atropine	as above	16	−	1.9±0.2
Ionophore A23187	1×10^{-6} M	8	‡	
Ionophore A23187	2×10^{-6} M	17	‡	
Ionophore A23187	5×10^{-6} M	27	‡	

Isotopically labelled exponentially growing cells were plated into adhesion medium containing the compounds at the concentrations indicated, and the initial rates of adhesion assayed as described in Fig. 1. The data are presented as the % of adherent cells after 30 min incubation. Neurite extension was scored as positive (+) if >50% of the cells had neurites in three days using the test conditions described in Fig. 2. For $^{45}Ca^{2+}$ flux assays, cells were plated on polylysine-coated 35-mm tissue culture dishes. The medium for the assay contained 150 mM NaCl, 2 mM $CaCl_2$, 5 mM KCl, 5 mM glucose, and 50 mM HEPES adjusted to *p*H 7.4 (ref. 13). $^{45}Ca^{2+}$ was included at 2 μCi ml^{-1}. $^{45}Ca^{2+}$ uptake was measured at 37°C by aspirating the growth medium and adding the assay medium containing the indicated compounds. Uptake was terminated at various times by aspirating the assay medium and washing the dishes quickly three times with nonradioactive assay buffer. Cells were dissolved in 0.2 M NaOH and the $^{45}Ca^{2+}$ content determined by scintillation counting. By measuring several time points the initial rate of $^{45}Ca^{2+}$ uptake could be determined[13]. The results are presented as ratios of the experimental rates to the control rate plus or minus the standard deviation of quadruplicate determinations.

* Cells incubated with cholera toxin for four hours before adhesion assay.

† Cells in these conditions became extremely flattened but did not extend neurites. Ultrastructual analysis of these cells will be presented elsewhere.

‡ Cytotoxic for extended incubations.

effect of NGF and cyclic AMP on calcium efflux of PC12 cells is similar to that of insulin on skeletal muscle, where insulin is thought to increase the free cytoplasmic calcium pool[14].

The effect of NGF and membrane depolarisation on lectin agglutination

NGF and cyclic AMP induced adhesion of PC12 cells to their substratum is rapid, energy dependent, and does not require protein synthesis[10]. These data suggest that an alteration of the cell surface by NGF leads to increased adhesion. One method of exploring this alternative is by examining the lectin-induced aggregation of cells, which is known to vary with the topographical distribution of glycoproteins on the cell surface and with membrane fluidity. By examining the agglutinability of PC12 cells by lectins with different specificities, it may be possible to detect alterations in the cell surface by the conditions which enhance adhesion. As NGF itself aggregates PC12 cells[10], it was necessary to measure the agglutination of the cells by lectins after preincubation with NGF. Cells were therefore incubated with NGF for one hour in serum-free HEPES medium, dissociated by pipetting, and the different lectins and their sugar antagonists added. They were then placed on a rotary shaker and aggregation assayed 20 min later by counting single cells on a Coulter counter[15]. The data in Table 3 show that phytohaemagglutinin (PHA) and ricin, with specificities for *N*-acetyl-D-galactosamine and galactose, respectively, agglutinate PC12 cells to a greater extent after exposure to NGF. On the other hand wheat germ agglutinin (WGA) and concanavalin A (Con A), with specificities for *N*-acetyl-D-glucosamine, and α-methylmannoside, respectively, did not distinguish NGF-treated cells from control cultures. *Ulex europeus* lectin, with a sugar specificity for fucose, did not agglutinate the cells in either condition.

Although these data are suggestive of an NGF-directed alteration in membrane properties, a kinetic analysis of the adhesion process was required. Because two aggregation processes are being examined, those influenced by NGF and by lectin, it was necessary to establish that two lectins with different specificities can alter the aggregation kinetics of NGF-treated control cultures differently. This would rule out any artefacts caused by nucleation phenomena in NGF-treated cells. PHA, which preferentially agglutinates NGF-treated cells, and WGA, which agglutinates both equally (Table 3), were studied. Cells were preincubated with NGF, 50 mM potassium, or cyclic AMP plus theophylline for one hour in 10% fetal calf serum. The cells were then dissociated by pipetting, and diluted into aliquots of HEPES medium containing PHA (10 μg ml^{-1}) or WGA (50 μg ml^{-1}), and the disappearance of single cells measured as a function of time. Figure 4 shows that the extent of agglutination induced by PHA is greater for NGF-treated cells than that caused by WGA. The agglutination data for cyclic AMP were quantitatively similar to those obtained with NGF (data not shown). The cells treated with elevated potassium, however, were agglutinated slightly better by WGA than PHA. This may be a reflection of the

different adhesive behaviour of potassium-treated cells, for although 50 mM potassium enhances cell–substratum adhesion as does NGF, it does not induce homotypic cell aggregation.

The above results could be explained in two ways: that there is a redistribution of the cell surface receptors following exposure to NGF which favours aggregation, or that there is a change in the number of surface receptors. To distinguish between these alternatives, PHA was isotopically labelled with ^{125}I and the number of binding sites on cells treated with NGF, cyclic AMP or 50 mM potassium determined. Table 4 shows that when cells were treated at room temperature with the above reagents and the number of PHA binding sites quantitated either at 4°C or 22°C, there was no significant change in the number of lectin receptors.

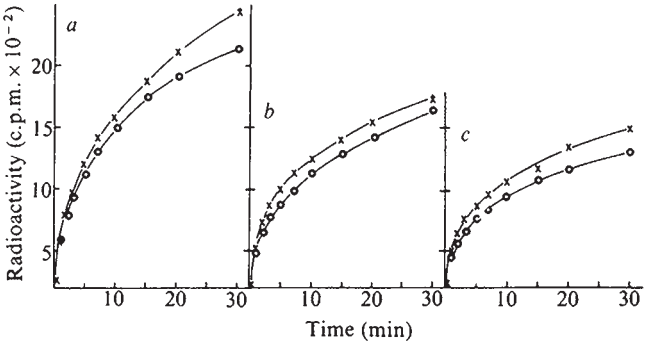
Conclusion

The above data suggest that NGF induces an increase in the intracellular level of cyclic AMP, causing an increase in calcium mobilisation, which in turn produces a structural change in the limiting cell membrane. This membrane alteration enhances cell–substratum adhesion and neurite extension in the PC12 cell line.

The following evidence suggests that cyclic AMP is a mediator of the NGF-induced responses of PC12 cells: (1) NGF elevates the level of endogenous cyclic AMP[10] (Table 2); (2) both NGF and cyclic AMP increase cell–cell and cell–substratum adhesiveness[10]; (3) they also increase the specific activity of choline acetyltransferase and intracellular acetylcholine accumulation[4]; (4) theophylline, a phosphodiesterase inhibitor, potentiates the effect of cyclic AMP and NGF (Table 1); (5) cholera toxin, which increases endogenous cyclic AMP accumulation[12], also promotes neurite outgrowth and adhesiveness (Table 1); (6) NGF and cyclic AMP lead to similar changes in total protein synthesis[16]. These biochemical data, plus the morphological and ultrastructural similarities of neurite outgrowth induced by dibutyryl cyclic AMP and NGF, strongly favour the role of cyclic AMP as a 'second message' in the NGF-responsive cell. The cyclic AMP-mediated adhesive and morphological response of cells is not unique for the PC12 cell line, for some C1300 neuroblastoma and central nervous system nerve cell clones extend processes in the presence of dibutyryl cyclic AMP[17,18], and cyclic nucleotides increase aggregation and cell–substratum adhesion in a variety of cell types[19,20].

Due to the calcium-dependent nature of cellular adhesion it is experimentally impossible to delete calcium from the culture

Fig. 3 Effect of NGF on calcium efflux. PC12 cells were grown at low density for two days in medium containing 2 μCi ml^{-1} $^{45}Ca^{2+}$. Identical numbers of these cells were transferred to polylysine-coated 60-mm tissue culture dishes and allowed to attach for 30 min in the presence of the $^{45}Ca^{2+}$. Efflux was initiated at 37°C by removing the radioactive medium, washing the dishes quickly three times with Ca^{2+}-free saline or Ca^{2+}-free saline containing either 800 units per ml of 7S NGF, 1 mM dibutyryl cyclic AMP, or 1 mM cyclic AMP plus 1 mM theophylline. 0.1-ml aliquots were removed at indicated times, centrifuged to remove any cells, and analysed for $^{45}Ca^{2+}$ content. Using a Wilcoxon signed ranks test for two related samples, P was less than 0.01 for these data. In addition to doing complete kinetic curves with one sample per point, triplicate determinations of Ca^{2+} efflux rates were made at a single time. The following are the results, c.p.m. ± s.d., at 30 min: control, 1,997 ± 7; 1×10^{-3} M cyclic AMP + 1×10^{-3} M theophylline, 2,372 ± 19; 3×10^{-5} M vinblastine, 2,152 ± 34; 800 units per ml NGF, 2,542 ± 45. These experiments were repeated at least three times with similar results. *a*, ×, NGF; ○, control. *b*, ×, cyclic AMP + theophylline; ○, control. *c*, ×, dibutyryl cyclic AMP; ○, control.



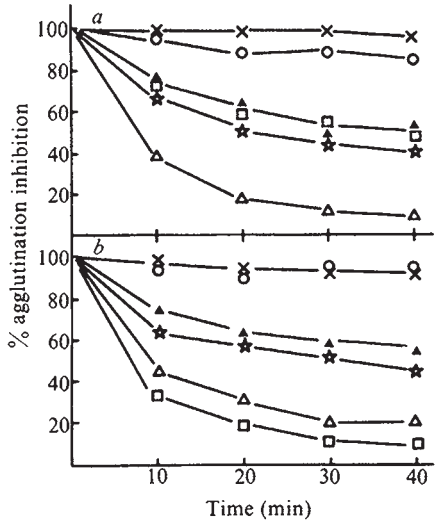


Fig. 4 NGF and potassium induced changes in lectin agglutination. Low-density exponentially dividing cells were washed twice in HEPES-medium containing 10% serum, and incubated with β-NGF (20 ng ml^{-1}), cyclic AMP (1×10^{-3} M) or potassium (50 mM, sodium replaced by potassium) in HEPES-medium with serum. The cells were then washed once in serum-free medium containing the various reagents, dissociated by pipetting, and pipetted into plastic tubes (0.5-ml aliquots at 1×10^{6} cells per ml) in serum-free HEPES-medium containing the compounds indicated below. The tubes were placed on a rotary shaker and the disappearance of single cells determined with a Coulter counter. The agglutination of cells by WGA (50 μg ml^{-1}) and PHA (10 μg ml^{-1}) was inhibited by >80% by 0.1 M *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine, respectively. The results with cyclic AMP are not shown, but were quantitatively similar to those with NGF. *a*, Cells preincubated with or without NGF. ×, control, no additive; ○, NGF alone; ▲, control plus WGA; □, NGF plus WGA; ★, control plus PHA; △, NGF plus PHA. *b*, Cells preincubated with or without 50 mM potassium. ×, control; ○, potassium; ▲, control plus WGA; ★, control plus PHA; △, potassium plus PHA; □, potassium plus WGA.

Table 2 Effect of several adhesion-promoting conditions on the intracellular level of cyclic AMP

Condition	Reagent concentration	Intracellular cyclic AMP (n)
Control	—	108 ± 19 (7)
β-NGF	20 ng ml^{-1}	176 ± 36 (9)
Potassium	50 mM	120 ± 40 (9)
Vinblastine	1×10^{-5} M	402 ± 40 (5)

Exponentially dividing cells were washed and incubated at 1×10^{6} cells per ml in 2 ml of HEPES-buffered medium plus 10% fetal calf serum for 30 min at 37°C. The various reagents were added to triplicate sets of cultures, the cells collected after 8 min by centrifugation, and cyclic AMP content determined[10]. The data are presented as the mean ± s.d. of n determinations. The differences between β-NGF and vinblastine samples and the control are highly significant ($P<0.01$), but that between potassium and control is not ($P=0.45$). Results are expressed as pmol cyclic AMP per mg cellular protein. The protein per cell was constant for all experimental samples.

Table 3 Agglutination caused by different lectins after exposure to NGF

Lectin	Concentration ($\mu g\ ml^{-1}$)	Competing sugar (0.2 M)	Agglutination Control	Agglutination NGF	Agglutination with sugar Control	Agglutination with sugar NGF
None	—	—	3	12	—	—
Ricin	1	Galactose	40	71	8	16
PHA	10	*N*-acetyl-D-galactosamine	50	85	7	12
Con A	5	α-methylmannoside	64	60	14	10
WGA	50	*N*-acetyl-D-glucosamine	30	32	5	10
Ulex	50	Fucose	6	15	5	17

Exponentially dividing cells were washed twice in serum-free HEPES medium, resuspended at 1×10^6 cells per ml, and either NGF (50 ng ml^{-1}) or saline added to 0.5-ml aliquots. One hour later the lectins or lectins plus 0.2 M sugar were added to cultures as indicated above and the cultures placed on a rotary shaker. Single cells were counted on a Coulter counter 20 min later. The data are presented as the % of the control cells at the time of lectin addition which are agglutinated. The experiments were repeated at least twice with similar results, and a kinetic analysis of some lectins is presented in Fig. 4.

medium. However, the following data suggest that the mobilisation of calcium, defined as increases in either net influx or efflux, is causally involved in the NGF mediated adhesive response and neurite extension in PC12 cells: (1) NGF and cyclic AMP increase calcium efflux (Fig. 3); (2) conditions which depolarise the cell result in an increase in calcium influx (Table 1). This is due to an activation of the voltage-dependent calcium channel in PC12 (ref. 13); (3) the calcium ionophore A23187 increases cell–substratum adhesion in a dose-dependent manner (Table 1); (4) potassium-induced adhesion is blocked by cobalt ions, which block the voltage-dependent calcium channel in PC12 cells (Table 1). This result established that membrane depolarisation per se is not responsible for the adhesive response. Thus, calcium is involved in the modulation of the PC12 cell–substratum adhesiveness and morphology.

It has become clear that calcium ions and the cyclic nucleotides are nearly ubiquitous and interrelated 'second messengers' in the activation of cells by defined extracellular molecules (see ref. 21 for review). In PC12 cells, it is likely that NGF first stimulates cyclic AMP synthesis, which in turn leads to an increase in calcium release from intracellular stores. As high external potassium leads to calcium mobilisation but does not increase intracellular cyclic AMP, it is likely that calcium mobilisation is both necessary and sufficient to elicit the adhesive and neurite outgrowth response. Increased intracellular cyclic AMP is apparently sufficient to induce these responses, but not necessary.

It should be pointed out, however, that the effects of conditions which lead to elevated intracellular cyclic AMP or calcium mobilisation alone are not identical. For example, the lectin agglutinability of cells exposed to 50 mM potassium is distinct from that of cells treated with NGF or cyclic AMP (Fig. 4). Assuming that calcium mobilisation is shared, these additional effects could be due to the cyclic nucleotide or changes associated directly with membrane depolarisation. If the regulation of phenotypic expression is under the control of several environmental stimuli, it may be that similar but distinct alterations are triggered individually by cyclic AMP, calcium and membrane depolarisation.

Once the apparent increase in 'free' intracellular calcium is achieved, either by influx or release from intracellular stores, then the ion must cause, or select for, an alternative membrane structure which leads to increased cell–cell and cell–substratum adhesion. Calcium-promoted changes have recently been described in erythrocyte membranes[22], and calcium regulates many aspects of membrane metabolism[21].

Changes in lectin agglutinability associated with cellular differentiation have been described in another clonal cell line[23]. During the induced erythroid differentiation of erythroleukaemic Friend cells, there is an initial increased agglutinability by a variety of lectins within a constant lectin receptor density[23]. Thus, in at least two clonal cell systems the initial events associated with developmental changes are accompanied by structural changes in the plasma membrane. The enhanced agglutinability of cells in the absence of increased receptor density may be due to an increased proximity of lectin receptors, resulting from either increased receptor mobility or from alterations in membrane morphology such as the formation of microvilli. Observations with the scanning electron microscope show no increase in microvilli formation after exposure to NGF or cyclic AMP (M.L., unpublished observation), suggesting that enhanced adhesion and neurite formation in PC12 cells are correlated with a redistribution or increased mobility of surface glycoproteins.

In addition to giving some insight into the mode of action of NGF, the above observations can be related to at least three additional areas. The first is the alteration of cell surface properties associated with membrane depolarisation and increased calcium flux. As PC12 cells release neurotransmitters in response to external stimuli[5,6], they can be viewed as being analogous to nerve terminals. Enhanced calcium permeability is a prerequisite for neurotransmitter release at nerve terminals, and the above data show that changes in membrane structure may accompany this process. Similar changes have been postulated to play a part in the development of the nervous system and may be involved in the 'stabilisation' of synaptic contacts once they are formed. Second, alterations in cell–cell adhesive properties certainly have key roles during embryogenesis, and the above data suggest that NGF-induced modification of membrane structure can trigger specific changes in cellular adhesion. For example, NGF can serve as a trophic factor, attracting neurite outgrowth from distant parts

Table 4 Binding of ^{125}I-PHA to cells

Condition	Concentration	4°C	22°C
Control	—	16,400±700	32,300±2,200
NGF	50 ng ml^{-1}	18,000±700	33,800±4,000
Cyclic AMP	1×10^{-3} M	18,700±1,200	37,600±3,800
Potassium	50 mM	20,800±1,400	29,300±3,000
Molecules of PHA bound per cell (control)		1.3×10^6	2.9×10^6

Binding of radioiodinated PHA. Purified PHA was labelled with ^{125}I in the presence of 0.1 M saccharide inhibitor[30], and the unbound iodine was removed by dialysis. The specific activity was 2.5×10^5 c.p.m. per μg protein and no change in agglutination activity was detected after iodination. For labelling, the cells were washed in serum-free HEPES medium and adjusted to 10^6 cells per ml in HEPES medium containing 0.5% bovine serum albumin (BSA). They were then incubated for one hour at 22°C in β-NGF, cyclic AMP, or K^+ at the concentrations indicated. 0.25 ml of cells were mixed with saturating concentrations of ^{125}I-PHA (20 μg ml^{-1}) or ^{125}I-PHA + 0.1 M *N*-acetyl-D-galactosamine, and incubated at 5°C or 22°C for 15 min. Cell pellets were counted after washing twice by centrifugation at the same temperature with HEPES + 0.5% BSA. The data are presented as c.p.m. ^{125}I-PHA specifically bound per 2.5×10^5 cells at the two assay temperatures, ±s.d. of three determinations. The nonspecific binding was 40–50% of the total.

of the nervous system[24]. As NGF reversibly increases cell–substratum adhesion, growing axons could be 'guided' by the NGF-induced increase in the adhesiveness of the growth cone to the underlying cells, rather than the growth cone following a substratum-associated gradient. Growth cones preferentially elongate on surfaces to which they adhere more firmly[25], and it should make no difference if the growth cone or substratum is reponsible for initiating the adhesive response. This type of NGF effect on cell–substratum adhesiveness could also explain the requirement for local exposure to NGF for continued neurite outgrowth in cultured sympathetic ganglion cells[26].

Finally, a similarity between three groups of clonal cell lines which are developmentally responsive to external stimuli should be noted. The cells are PC12, C1300 mouse neuroblastoma and the Friend erythroleukaemic lines. As outlined above, NGF, cyclic AMP and compounds which induce calcium mobilisation in PC12 cells lead to developmental changes. In C1300 neuroblastoma cells there is a well defined relationship between cell–substratum adhesiveness and morphological differentiation[9] and such compounds as cyclic AMP, butyric acid, dimethyl sulphoxide (DMSO), prostaglandin E_1, acetylated diamines and ouabain (D.S., unpublished observation) also induce neurite extension[9,27]. A set of reagents overlapping the above also induce erythroid differentiation in Friend virus-transformed erythroleukaemic cells. These include DMSO, short-chain fatty acids, polymethylene acetamide, prostaglandin E_1, and ouabain (see ref. 28 for discussion). Although it is extremely difficult to envisage a direct action of all these diverse conditions and reagents on the genome of the cells, it is likely that all interact to varying extents with the plasma membrane. It has, for example, been demonstrated that DMSO, divalent ions, and other cryoprotective agents produced an increase in the phase transition temperature of membranes which correlated with their inductive effects on erythroid differentiation[29]. Thus, it is likely that all of these reagents produce their phenotypic effects by initially altering the membrane structure, perhaps by causing a change in calcium ion permeability. The requisite next step would be the flow of this information acquired by the membrane to the biosynthetic machinery of the cell. The mechanism of this transduction remains to be described.

β-NGF and its antiserum were obtained from R. Goodman and H. Herschman. Supported by the Muscular Dystrophy Association.

Received 9 December 1977; accepted 24 April 1978.

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Sequence of chicken ovalbumin mRNA

L. McReynolds*, B. W. O'Malley*, A. D. Nisbet†, J. E. Fothergill†, D. Givol‡, S. Fields§, M. Robertson§ & G. G. Brownlee§

*Baylor College of Medicine, Houston, Texas
†Department of Biochemistry, University of Aberdeen, Aberdeen, UK
‡The Weizmann Institute, Rehovoth, Israel
§MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

The complete sequence of chicken ovalbumin mRNA is presented; it is 1,859 residues long, excluding its terminal 'cap' and poly(A). The region coding for ovalbumin lies close to the 'cap' but is separated from the poly(A) by an extensive 3′ noncoding region of 637 nucleotides which may have no function that is precisely dependent on its sequence.

CHICKEN ovalbumin and its gene provide a favourable and interesting model system for studying the regulation of gene action by hormones[1,2]. It is firmly established[3] that steroid hormones will switch on the expression of all the egg proteins, including ovalbumin, and that the hormone probably acts at the DNA level through a steroid receptor protein. However, the detailed molecular mechanism and details of the specific site or sites involved on the DNA are unknown.

As a first approach to understanding the basic molecular biology of this gene we decided to sequence the messenger RNA. Since we started this work it has become clear that the ovalbumin gene[4–7], as well as other genes such as the β-globin[8,9] and immunoglobulin genes[10] are interrupted by 'introns'[11] or inserted sequences. This complication was unexpected and made it essential that an accurate sequence of the mRNA be established for future comparison with gene sequences.

Previous sequencing of ovalbumin mRNA has been limited to 130 residues immediately adjacent to the poly(A), with a further tentative sequence from 131 to 191 residues from the poly(A)[12,13]. This study revealed the presence of the sequence A-A-U-A-A-A centred about 20 residues away from the poly(A)[14], although the function of this signal remains unknown.

The sequence work described here was possible because of the synthesis and isolation of an almost full length duplex DNA copy of the mRNA[15] and its subsequent cloning in the

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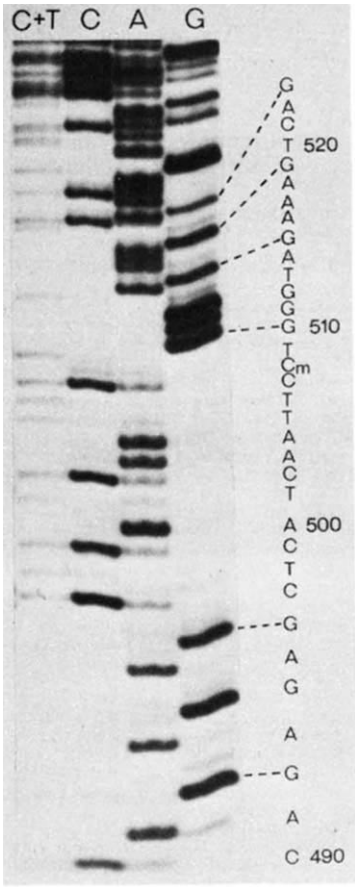

Fig. 1 Sequence derived from residues 490–523 from *Pst*-labelled *Hae*III-recut 341-long fragment. G and A refer to the 'alternate G' and 'alternate A' procedures, whilst C and C+T are the standard hydrazine procedures[18]. Residue 508 is methylated as it is part of an *Eco* RII recognition site, and the resultant 5-methyl C (Cm) appears as a gap, or very weak C. 40 μg of pOv230 was opened as its sole *Pst*I site by restriction with 60 units *Pst*I (New England Biolabs) for 1 h at 37 °C in a volume of 200 μl. An equal volume of phenol was added and, after vortex mixing and centrifugation to separate the two layers, the linear DNA was precipitated from the aqueous layer by the addition of one-tenth volume of 3 M sodium acetate and 3 volumes of ethanol. The DNA was treated with excess bacterial alkaline phosphatase (Sigma) (20 μg) in the presence of venom phosphodiesterase (0.5 μg) in 0.01 M Tris-acetate 0.01 M $MgCl_2$, *p*H 8.5 in a 100 μl reaction for 30 min at 37 °C. The enzymes were removed and the DNA precipitated by a similar phenol inactivation to that described above for the restriction enzyme. Labelling was carried out using 200 μCi γ-^{32}P-ATP (3,000–5,000 Ci $mmol^{-1}$, Radiochemical Centre, Amersham) in a volume of 25 μl using 5–20 units of T4-polynucleotide kinase (P.L. Biochemicals or Boehringer) in 1×glycine–NaOH buffer, *p*H 9.5 (2×buffer is 100 mM glycine-NaOH, *p*H 9.5, 20 mM $MgCl_2$, 10 mM dithiothreitol, 0.2 mM spermidine, 50% glycerol) for 30 min at 37 °C. The total reaction mixture, after the addition of 20 μl H_2O and 0.5 μl of 10 mM ATP, was treated with phenol to remove enzyme and the labelled DNA precipitated, as before, in the presence of 0.3 M ammonium acetate. Recutting was with 100 units of *Hae*III (New England Biolabs) for 1 h at 37 °C in a volume of 20 μl, after which 5 μl of a 30% sucrose solution containing marker dyes (bromophenol blue and xylene cyanol FF) was added. The sample was loaded onto a 3 mm×1.5 mm well of a nondenaturing 4% and 40 cm long acrylamide gel in Tris–borate–EDTA buffer[18] and run for 6 h at 400 kV. The faster band running close to the xylene cyanol FF (slow) dye was eluted[18] and subjected to the Maxam and Gilbert procedure and fractionation on a 20% acrylamide gel. Sensitive autoradiography with phosphotungstate screens at −80 °C was used to detect bands[40].

Escherichia coli plasmid pMB9[16] using the poly(A)/poly(T) tailing method. Plasmids were screened for the size of inserted sequence and the longest clone (named pOv230) containing about 1,850 residues of ovalbumin mRNA was selected for sequencing. Similar plasmids have been isolated by other workers[17].

Ovalbumin sequences in clone pOv230

Clone pOv230 was sequenced by the chemical degradation method of Maxam and Gilbert[18]. An initial difficulty was that there was no satisfactory method of excising the inserted ovalbumin sequence from the vector plasmid. This problem was overcome in one approach by the isolation in bulk of two large *Hae*III restriction fragments which contained mainly ovalbumin-derived sequences. Another approach was to radioactively label a restriction enzyme digest of the entire pOv230 and then purify the specific ovalbumin-containing fragments. Full details of the sequence analysis will be published elsewhere, but as an example, the sequence established by labelling the single *Pst*I site in the intact pOv230 is shown in Fig. 1. Similar labelling of restriction fragments by six different restriction enzymes known to cut ovalbumin sequences[15], that is, *Hae*III, *Hin*fI, *Alu*I, *Mbo*II, *Hph*I and *Hga*I, allowed the entire ovalbumin-derived part of the plasmid to be sequenced (residues 13–1,859 of Fig. 2). A computer search [19] of this sequence showed the final positions (see Table 1) of cutting sites, not only for those enzymes used in the sequence analysis, but also of all other presently known restriction enzymes. Considering the large number of sites, there was fair agreement with the previously published restriction map of ovalbumin cDNA[15] although additional *Xba*I, *Pvu*II and *Taq*I sites were established and more sites were present for some of the enzymes than was previously thought.

Sequence accuracy

The sequence of the coding region of the mRNA (residues 65–1,222) was considerably helped by a comparison of the nucleotide sequence with known amino acid sequence. Several selected regions of amino acid sequence such as those of the N

Table 1 Known restriction recognition sites in pOv 230 (residues 13–1,859 of Fig. 1)*

Enzyme	Residue nos†	Sequence
*Pst*I	477	CTGCA/G
*Hae*III	818	GG/CC
*Hin*fI	258, 566, 1458, 1732	G/ANTC
*Hga*I	1125	GCGTC
*Hph*I	60, 336, 1605	TCACC
*Alu*I	15, 38, 117, 175, 277, 475, 495, 557, 759, 828, 986, 1042, 1235, 1332, 1338, 1554	AG/CT
*Mbo*II	147, 308, 389, 603, 638, 748, 887, 895, 922, 989, 1030, 1133, 1161, 1197, 1229, 1669	GAAGA or TCTTC
*Eco*RII‡	193, 213, 254, 507, 754	/CC(A/T)GG
*Mbo*I‡	482, 751, 898	/GATC
*Taq*I	41	T/CGA
*Xba*I	1345	T/CTAGA
*Sst*I	116, 494	GAGCT/C§
*Pvu*II	474	CAG/CTG
*Asu*I	867	G/GACC
*Mnl*I	325, 444, 447, 892, 914, 929, 943, 1014, 1085, 1103, 1159	CCTC or GAGG

* No sites for *Hae*II, *Hind*II and III, *Hha*I, *Hpa*I and II, *Eco*RI, *Ava*I, *Bam*I, *Bal*I, *Bgl*II, *Sma*I, *Xho*I and *Sal*I.

† The number given is the first (5′) nucleotide of the recognition sequence and not the point of cutting. Where this is within the recognition sequence it is indicated by a vertical bar.

‡ These enzymes do not cut in pOv230 grown in *E. coli* strain RR1[42] because of methylation of the C (*Eco*RII) or the A (*Mbo*I) residues.

§ Recognition sequence established by C. D. Liarakos (personal communication). For others see ref. 43.

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mG
 '                                                                   M  G  S  I  G  A  A  S  M  E  F  C  F  D  V  F  K  E  L
ACAUACAGCUAGAAAGCUGUAUUGCCUUUAGCAGUCAAGCUCGAAAGACAACUCAGAGUUCACCAUGGGCUCCAUCGGUGCAGCAAGCAUGGAAUUUUGUUUUGAUGUAUUCAAGGAGCU
        10        20        30        40        50        60        70        80        90       100       110       120

 K  V  H  H  A  N  E  N  I  F  Y  C  P  I  A  I  M  S  A  L  A  M  V  Y  L  G  A  K  D  S  T  R  T  Q  I  N  K  V  V  R
CAAAGUCCACCAUGCCAAUGAGAACAUCUUCUACUGCCCCAUUGCCAUCAUGUCAGCUCUAGCCAUGGUAUACCUGGGUGCAAAAGACAGCACCAGGACACAAAUAAAUAAGGUUGUUCG
       130       140       150       160       170       180       190       200       210       220       230       240

                            (P)
 F  D  K  L  P  G  F  G  D  S  I  E  A  Q  C  G  T  S  V  N  V  H  S  S  L  R  D  I  L  N  Q  I  T  K  P  N  D  V  Y  S
CUUUGAUAAACUUCCAGGAUUCGGAGACAGUAUUGAAGCUCAGUGUGGCACAUCUGUAAACGUUCACUCUUCACUUAGAGACAUCCUCAACCAAAUCACCAAACCAAAUGAUGUUUAUUC
       250       260       270       280       290       300       310       320       330       340       350       360

 F  S  L  A  S  R  L  Y  A  E  E  R  Y  P  I  L  P  E  Y  L  Q  C  V  K  E  L  Y  R  G  G  L  E  P  I  N  F  Q  T  A  A
GUUCAGCCUUGCCAGUAGACUUUAUGCUGAAGAGAGAUACCCAAUCCUGCCAGAAUACUUGCAGUGUGUGAAGGAACUGUAUAGAGGAGGCUUGGAACCUAUCAACUUUCAAACAGCUGC
       370       380       390       400       410       420       430       440       450       460       470       480

 D  Q  A  R  E  L  I  N  S  W  V  E  S  Q  T  N  G  I  I  R  N  V  L  Q  P  S  S  V  D  S  Q  T  A  M  V  L  V  N  A  I
AGAUCAAGCCAGAGAGCUCAUCAAUUCCUGGGUAGAAAGUCAGACAAAUGGAAUUAUCAGAAAUGUCCUUCAGCCAAGCUCCGUGGAUUCUCAAACUGCAAUGGUUCUGGUUAAUGCCAU
       490       500       510       520       530       540       550       560       570       580       590       600

 V  F  K  G  L  W  E  K  A  F  K  D  E  D  T  Q  A  M  P  F  R  V  T  E  Q  E  S  K  P  V  Q  M  M  Y  Q  I  G  L  F  R
UGUCUUCAAAGGACUGUGGGAGAAAGCAUUUAAGGAUGAAGACACACAAGCAAUGCCUUUCAGAGUGACUGAGCAAGAAAGCAAACCUGUGCAGAUGAUGUACCAGAUUGGUUUAUUUAG
       610       620       630       640       650       660       670       680       690       700       710       720

 V  A  S  M  A  S  E  K  M  K  I  L  E  L  P  F  A  S  G  T  M  S  M  L  V  L  L  P  D  E  V  S  G  L  E  Q  L  E  S  I
AGUGGCAUCAAUGGCUUCUGAGAAAAUGAAGAUCCUGGAGCUUCCAUUUGCCAGUGGGACAAUGAGCAUGUUGGUGCUGUUGCCUGAUGAAGUCUCAGGCCUUGAGCAGCUUGAGAGUAU
       730       740       75Q       760       770       780       790       800       810       820       830       840

                                                                                                            CHO
                                                                                                             '
 I  N  F  E  K  L  T  E  W  T  S  S  N  V  M  E  E  R  K  I  K  V  Y  L  P  R  M  K  M  E  E  K  Y  N  L  T  S  V  L  M
AAUCAACUUUGAAAAACUGACUGAAUGGACCAGUUCUAAUGUUAUGGAAGAGAGGAAGAUCAAAGUGUACUUACCUCGCAUGAAGAUGGAGGAAAAAUACAACCUCACAUCUGUCUUAAU
       850       860       870       880       890       900       910       920       930       940       950       960

 A  M  G  I  T  D  V  F  S  S  S  A  N  L  S  G  I  S  S  A  E  S  L  K  I  S  Q  A  V  H  A  A  H  A  E  I  N  E  A  G
GGCUAUGGGCAUUACUGACGUGUUUAGCUCUUCAGCCAAUCUGUCUGGCAUCUCCUCAGCAGAGAGCCUGAAGAUAUCUCAAGCUGUCCAUGCAGCACAUGCAGAAAUCAAUGAAGCAGG
       970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080

                (P)
 R  E  V  V  G  S  A  E  A  G  V  D  A  A  S  V  S  E  E  F  R  A  D  H  P  F  L  F  C  I  K  H  I  A  T  N  A  V  L  F
CAGAGAGGUGGUAGGGUCAGCAGAGGCUGGAGUGGAUGCUGCAAGCGUCUCUGAAGAAUUUAGGGCUGACCAUCCAUUCCUCUUCUGUAUCAAGCACAUCGCAACCAACGCCGUUCUCUU
      1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200

 F  G  R  C  V  S  P  *
CUUUGGCAGAUGUGUUUCCCCUUAAAAAGAAGAAAGCUGAAAAACUCUGUCCCUUCCAACAAGACCCAGAGCACUGUAGUAUCAGGGGUAAAAUGAAAAGUAUGUUAUCUGCUGCAUCCA
      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320

GACUUCAUAAAAGCUGGAGCUUAAUCUAGAAAAAAAAUCAGAAAGAAAUUACACUGUGAGAACAGGUGCAAUUCACUUUUCCUUUACACAGAGUAAUACUGGUAACUCAUGGAUGAAGGC
      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430      1440

UUAAGGGAAUGAAAUUGGAGUCAUCACACUGAAAAAUGCAACCUGAUACAUCAGCAGAAGGUUUAUGGGGGAAAAAUGCAGCCUUCCAAUUAAGCCAGAUAUCUGUAUGACCAAGCUGCU
      1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560

CCAGAAUUAGUCACUCAAAAUCUCUCAGAUUAAAUUAUCAACUGUCACCAACCAUUCCUAUGCUGACAAGGCAAUUGCUUGUUCUCUGUGUUCCUGAUACUACAAGGCUCUUCCUGACUU
      1570      1580      1590      1600      1610      1620      1630      1640      1650      1660      1670      1680

CCUAAAGAUGCAUUAUAAAAAUCUUAUAAUUCACAUUUCUCCCUAAACUUUGACUCAAUCAUGGUAUGUUGGCAAAUAUGGUAUAUUACUAUUCAAAUUGUUUUCCUUGUACCCAUAUGU
      1690      1700      1710      1720      1730      1740      1750      1760      1770      1780      1790      1800

AAUGGGUCUUGUGAAUGUGCUCUUUUGUUCCUUUAAUCAUAAUAAAAACAUGUUUAAGC - Poly A
      1810      1820      1830      1840      1850
```

Fig. 2 Sequence of ovalbumin messenger RNA. Residues 13–1,859 were present in the plasmid pOv230: residues 1–12 and 1,731–1,859 were sequenced directly from the mRNA. * Marks the terminator, Ⓟ the serine phosphate residues, and 'CHO' the carbohydrate. Mature ovalbumin lacks the N-terminal methionine; the resultant N-terminal glycine is *N*-acetylated. The amino acid composition, excluding the initiator Met, is Ala (A), 35; Arg (R), 15; Asn (N), 17; Asp (D), 14; Cys (C), 6; Gln (Q), 15; Glu (E), 33; Gly (G), 19; His (H), 7; Ile (I), 25; Leu (L), 32; Lys (K), 20; Met (M), 16; Phe (F), 20; Pro (P), 14; Ser (S), 38; Thr (T), 15; Trp (W), 3; Tyr (Y), 10 and Val (V), 31. Total, 385 residues.

terminus[20], the C terminus[21], the carbohydrate linkage[22], the sites of serine phosphorylation[23], and the region of a genetic variant[24], have been published, but represent only a small percentage of the total. However, almost complete peptide sequence information from trypsin and cyanogen bromide cleavage has been established as part of the determination of the complete amino acid sequence of the ovalbumin protein (A.D.N. *et al.*, in preparation). The independent nature of the evidence from both nucleotide and amino acid sequencing acted as a powerful check on the accuracy of the sequence. As a further check on accuracy about two-thirds of the coding region was sequenced on both strands of the DNA of the plasmid. In the noncoding regions the sequence was determined on both strands of the DNA for all but 23 residues, where it proved technically difficult. Sequencing each strand of the DNA is a valuable confirmation of the sequence and is probably essential for 100% accuracy.

In addition to the above checks care was taken to overlap the sequence data derived from different restriction fragments in the noncoding region and also in the coding region if the amino acid sequence was uncertain. In the coding region no overlap was sought across the fragments labelled at the *Pst*I site (residue 477) or the *Alu*I site (1,042), because the amino acid sequence was reliable. By contrast, an *Alu*I fragment overlapping the *Hae*III site (818) was sequenced because here the amino acid sequence was not established. In the noncoding

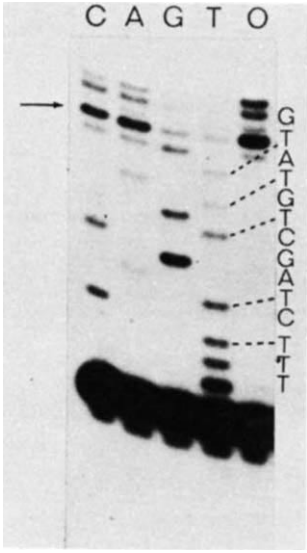

Fig. 3 Sequence of 5′ end of mRNA by 'primed synthesis'. C, A, G and T refer to the four separate dideoxynucleoside triphosphate reactions. O is a control containing all four deoxynucleoside triphosphates but with no added dideoxynucleoside triphosphates. The arrow marks the major 'full stop' band while the intense band at the bottom is unused primer. The primer was a ^{32}P-end-labelled 23-long *Alu*I fragment (residues 17–39) prepared by ^{32}P-end-labelling an *Alu*I digest of 40 μg of pOv230 using a protocol identical to that used for labelling the *Pst*I site (see legend to Fig. 1), except that no venom phosphodiesterase was used. After the phosphokinase labelling in 22 μl, 5 μl of the 30% sucrose dye solution was added and the samples loaded directly onto a 1.5 mm×25 mm slot of a 40 cm long 12% nondenaturing acrylamide gel in 1×Tris–borate–EDTA buffer[12] and electrophoresis carried out for 16 h at 200 V. The 23-long *Alu*I fragment ran close to the fast blue dye and was identified by its absence from a control, but otherwise identical digest and labelling, of pMB9; it was eluted in the presence of 20 μg carrier tRNA, but excluding sodium dodecyl sulphate. Before setting up the 'dideoxy' reactions, a tenth of the fragment (2 μl) was boiled with 20 μl water for 2 min to denature it and then mixed with 25 μl of 1.5 mg ml^{-1} of partially purified ovalbumin mRNA (from S. L. Woo). 4 μl of this primed mRNA was used in a 10 μl reaction volume containing all four deoxynucleoside triphosphates at 0.02 mM and the relevant single dideoxynucleoside triphosphate (from F. Sanger) at 0.1 mM with buffer[12] and reverse transcriptase (from W. Beard). After 45 min at 37 °C, 3 μl of a 90% formamide, 0.02 mM EDTA solution containing dyes was added and the sample boiled for 5 min in an open tube to reduce the volume to 3 μl, which was loaded onto a 5 mm wide slot of a 40 cm long 12% thin gel[41] and electrophoresed at 1.3 kV for 1.5 h.

region only the *Hin*fI site (1,458) was not overlapped by sequencing an independent restriction fragment. However, in this case the internal 'N' nucleotide of this '5′ overhanging' restriction site (G-A-N-T-C) was sequenced on both strands and provided the overlap. This observation made it unlikely that there were two *Hin*fI sites very close together.

The sequence near the poly(A) region was less extensively checked because residues 1,730–1,859 had been sequenced previously using 'primed synthesis' on the mRNA directly[12]. However, we established that pOv230 included all the known poly(A)-adjacent sequences of the mRNA by sequencing a *Hin*fI fragment labelled at residue 1,732. The tentative sequence previously proposed for the region of 131–191 residues from the poly(A) was corrected (nine changes), eliminating two postulated restriction sites. Thus no apparent mutation or rearrangement of sequences occurred during cloning so that the plasmid represents an exact copy of the mRNA. Despite the care taken to ensure accuracy the possibility of occasional errors remains.

Sequence at the 5′ end of the mRNA

The sequence of pOv230 extended into the 5′ noncoding region, but there was no way of assessing from the sequence itself how close it was to the 5′ end of the mRNA. These additional 12 nucleotides were defined by using primed synthesis on the mRNA template with reverse transcriptase. Similar methods have been used to define the 5′ noncoding regions of globin mRNA[25]. A chain terminator method[26] (P. Hamlyn *et al.*, in preparation) using dideoxynucleoside triphosphates was used to define this sequence (Fig. 3). Residue 1 (an A) was deduced from a 'partial minus' reaction[25] (results not shown). In these various reactions with reverse transcriptase some minor synthesis invariably occurred for 1–3 residues beyond the major 'full-stop'[25] position. We have not established whether this represents a length microheterogeneity at the 5′ end of the mRNA, or whether it reflects errors in copying due to the presence of the cap structure. The presence of the m^{7}Gppp cap structure was assumed from translation studies[27]. We have not assessed whether N^6 methylation occurs at residue 1 as in the rabbit globin mRNAs[28].

Significance of the mRNA sequence

The 1,859 residue long ovalbumin mRNA is the third eukaryotic mRNA to be completely sequenced and makes an interesting comparison with the completed rabbit[29] and human[25,30] β-globin sequence as well as the partial sequence available for α-globin[25], immunoglobulin (P. Hamlyn *et al.*, in preparation) and other mRNAs. Like all eukaryotic mRNAs it has more nucleotides than are needed to code for its specific protein, but the length of the 3′ noncoding region is remarkable. Specifically, ovalbumin mRNA has a 64-long 5′ noncoding region, a 1,158-long coding region, and a 637-long 3′ noncoding region. The 637 noncoding residues at the 3′ end are more extensive than the entire rabbit β-globin sequence (589 residues).

(1) *5′ Noncoding region.* This region is believed to be required for initiation of protein synthesis, but the molecular details of how this is achieved are unknown. The sequence is of comparable length to other 5′ noncoding sequences but is otherwise quite dissimilar in sequence. It seems that only the cap and A-U-G are conserved sequence features for all the 17 known 5′ noncoding sequences. This has led to the suggestion that apart from the cap, which is known to augment initiation, A-U-G itself is the only important general signal for initiation in higher organisms[44].

It is notoriously difficult to predict secondary structure models from primary sequence studies, but nevertheless a convincing 'hairpin loop' can be drawn close to the cap (Fig. 4). This is a highly stable structure[31] ($-\Delta G$ (25 °C)=11.4 kcal) and additional data for its existence was evident in the sequence determination of this region (Fig. 4 legend). A tight hairpin loop near the cap is not a conserved feature in all mRNAs, but it might have a special, if as yet unknown, significance for ovalbumin mRNA. However, we could speculate that this hairpin loop exists to bring the 'cap' and the A-U-G close together in the tertiary structure of the mRNA, despite the fact that these are 65 residues apart in the primary structure. The remainder of the 5′ noncoding region may also participate in local hairpin loops but these are of marginal stability and several alternatives are possible, which cannot yet be distinguished. It may be noted that there are no A-U-G triplets other than the initiation triplet in this region suggesting that the first A-U-G from the cap might specify initiation. But, as this is not true of other known 5′ noncoding sequences[44], the significance of this is doubtful.

(2) *Coding regions.* The entire mRNA sequence, even excluding the poly(A) region, is rich in A+U (A+U=58.1%) and the coding region reflects this (A+U=55.3%). This imposes no severe constraints on the amino acid sequence because of

redundancies in the genetic code. All codons are used at least once (Fig. 5) with the exception of C-C-G (proline), G-C-G (alanine) and C-G-U, C-G-A and C-G-G (arginine). These codons all possess the doublet C-G, which is rare in eukaryotic DNA[32] and in globin mRNA[29]. C-G doublets are not entirely excluded since there are 12 in the coding region. Nevertheless, this is a significantly lower number than is found for any of the other 15 doublets. The reason for the rarity of (5′) C-G (3′) as opposed to the other 15 possible doublets is not known but may be correlated with N^5 methylation of the cytosine residues in the DNA[33]. Other codons which are rarely used are C-U-A (once, leucine) and C-C-C (once, proline). Nonrandom use of codons has been noted before in bacteriophage ΦX DNA[34] and in rabbit β-globin mRNA[29] but the reason is again unknown. (3) *3′ Noncoding region.* This region is most obviously characterised by its length of 637 nucleotides including the U-A-A terminator, and is by far the longest known 3′ noncoding region. It compares, for example, with 95 residues in rabbit β-globin and 211 residues in a mouse κ immunoglobulin light chain (P. Hamlyn *et al.*, in preparation). A detailed examination of this sequence reveals several other features which distinguish it from the coding region. For example, 'runs' of a single base (for example A_5, A_8, G_5) are more frequent than would be expected for a random nucleotide sequence of equivalent overall composition and length. Another feature is the presence of short repeats or near repeats: G_4-U-A_4 (residues 1,285–1,293) is repeated with one base change as G_5-A_4 (1,507–1,515) and G-A_5-U-G-C-A is also exactly repeated (1,471 and 1,511). The A-rich sequence immediately following the U-A-A terminator is particularly striking and is repeated with one change in the A_8 sequence (1,351). There are many other shorter runs and repeats. There are no C-G doublets.

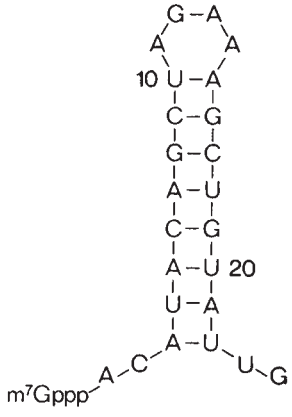

Fig. 4 Hairpin loop near cap. The sequence determinations in this region showed severe 'compression' (close spacing) at the T-G-T sequence when the acrylamide gel of Fig. 3 was run 'cold', that is at 250 V, 16 h on a standard 1.5 mm thick gel, rather than 'hot' as described in the legend to Fig. 3. 'Compression' invariably correlates with hairpin loop structures, and so provides positive evidence for their existence[12].

A comparison with the same region of other mRNAs reveals certain similar features which have not previously been emphasised. For example, rabbit α-globin mRNA[35] contains a C_8 sequence while the human α-globin contains three near repeats of the hexanucleotide G-G-G-C-C-C[25]. An immunoglobulin light chain mRNA also contains an unusual localised repeat based on the triplet U-C-C (P. Hamlyn *et al.*, in preparation). Moreover, longer areas covering 30 nucleotides of partial repeat sequence were noted in the human β-globin mRNA[36]. Simple repeat sequences are also characteristic of nontranscribed DNA under no selective pressure[37], such as parts of the 5S DNA 'spacer' of *Xenopus*[33] and also satellite DNA.

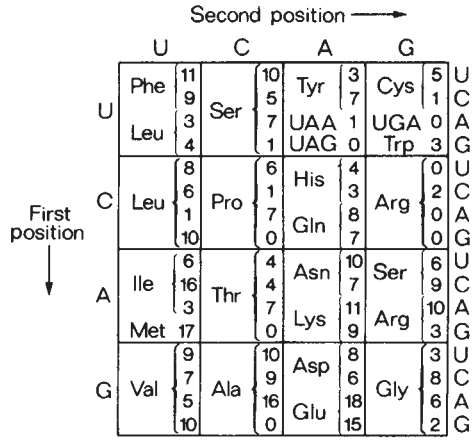

Fig. 5 Codon usage in ovalbumin mRNA.

The presence of these homopolymeric and repeat sequences suggests that the 3′ noncoding region of ovalbumin mRNA, and indeed other mRNAs, may not be a region with a precise sequence-dependent function. Rather these regions may have similar evolutionary origins through multiple repetitions of simple sequences. This could explain the variable length and sequence features in all of the known 3′ noncoding regions—a variability that would be surprising if special functions were to be ascribed to these regions. It is also possible that there is a limited sequence variation in this region of the mRNA which would not be revealed here as the sequence was determined mainly from a single clone. Despite this, there may be evolutionary constraints on the 3′ noncoding sequence of a particular mRNA because it has some property, for example the overall shape of the mRNA, which is not sequence-specific. This could explain the observed 80% homology of the noncoding regions in rabbit and human β-globin mRNA[36].

One sequence, the hexanucleotide A-A-U-A-A-A (residues 1,841–1,846) is conserved in different mRNAs and occurs about 20 residues away from the poly(A)[14] but its function remains unknown. Given the evolutionary explanation for the 3′ noncoding region and its position near the 3′ end of the mRNA, the strong possibility emerges that it is related to the termination of transcription or to a subsequent processing of the mRNA. However, A-A-U-A-A-A occurs elsewhere in the coding region of ovalbumin mRNA (223–228), suggesting that it alone may be a necessary but not a sufficient signal.

Protein sequence

The amino acid sequence predicted here is in complete agreement with an almost completed analysis using classical direct sequence analysis of ovalbumin (A.D.N. *et al.*, in preparation). It is clear, therefore, that the sequence shown above represents the major ovalbumin protein corresponding to one of the known genetic variants[24]. The other allelic form can be explained by a point mutation of residue 998 from A to G, giving an aspartic acid instead of an asparagine residue. Ovalbumin is both glycosylated and phosphorylated at the positions shown (Fig. 2), and the position of the single 'intrachain' disulphide bond is established in part[38].

Ovalbumin is a secreted protein. Most secreted proteins have a transient N-terminal hydrophobic region of sequence or 'signal peptide'[39] which is removed during the secretion process. But translation studies[20] have shown that ovalbumin lacks such a signal sequence. The nucleotide sequence that we have established substantiates this observation, as an A-U-G codon immediately precedes the triplet coding for the first amino acid in ovalbumin. Presumably a somewhat specialised mechanism exists for ovalbumin secretion.

We thank R. Staden for assistance with computer data storage and analysis and the MRC, the Baylor Center for Population Research and Reproductive Biology, and the Keasbey Memorial Foundation for financial support. D.G. was recipient of an EMBO fellowship.

Received 12 April; accepted 11 May 1978.

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letters to nature

A Near-Eastern sighting of the supernova explosion of 1054

ASTRONOMERS have long been puzzled by the apparent failure of observers in Europe and the Near East to see and report the Crab supernova of 1054. However, we now present evidence of such a sighting.

On 4 July 1054 a supernova explosion was observed and recorded in several reports by astronomers in China and Japan. For example, the report in the *Sung-shih* (Astronomical Treatise, Chapter 56) says, "First year of the Chih-ho reign period, fifth month, (day) *chi-ch'ou* (a 'guest star', that is, a nova) appeared approximately several inches (*k'o-shu-ts'un*) to the south-east of *T'ien-kuan* (ζ Tau). After a year and more it gradually vanished."[1,2] This remarkable observation was first brought to light in the west by Biot[3] in 1846. Lundmark[4] in his list of suspected new stars recorded in old chronicles, published in 1921, notes the proximity of this object to NGC1052—the Crab nebula. Although Duncan[5] measured the expansion rate of the filaments of the Crab in the same year (1921) and found that a simple linear extrapolation of their motion with constant velocity implied an origin about 900 years earlier, it was not until 1928 that Hubble[6] first drew the connection between the expansion rate and the Chinese observation to identify the Crab nebula with the 'guest star' observed by the Chinese in 1054. The subsequent historical researches[1,7,8] have mainly served to strengthen this identification (but see ref. 9 for some reservations).

The Chinese records indicate that the 'guest star' was brighter than Venus, giving it a probable visual magnitude of −4 or −5 at maximum. It was visible in the day time for 23 days, and continued to be visible in the night sky for perhaps 6 months. It is therefore puzzling that no reference to this event has been found in European or Arabic records of the time[6,10–12]. (A possible record in certain south-west Indian petroglyphs has been proposed by Miller[13], and further supported by Brandt[14]. However, see Ellis[15] and S. H. Koenig (in preparation) for some cautionary remarks.) This led to the conclusion, as expressed, for example, by the historian of science George Sarton, that "The failure of medieval Europeans and Arabs to recognise such phenomena was not due to any difficulty in seeing them, but to prejudice and spiritual inertia connected with the groundless belief in celestial perfection."[16] However, since they quite accurately reported the position and other properties of another supernova which occurred in 1006, only 48 years earlier[17], as well as many other astronomical phenomena, such as Halley's comet (witness the Bayeux tapestry), and meteorological phenomena, such as aurorae, this has never seemed to be a very convincing explanation. Others have suggested that the whole of the Middle East and Europe was clouded out for 6 months, an even more dubious proposition. We present here for the first time what we believe to be an eyewitness account of a sighting of the 1054 supernova explosion in the Near East.

The event and its supposed sequelae were mentioned by Ibn Buṭlān, a Christian physician of Bagdad, who lived in Cairo for 3 years until late in 1052 or early in 1053. Then, probably as the result of a famine, he left for Constantinople where he stayed for about a year. His original report was reproduced by Ibn Abī Uṣaybiʿa (hereafter referred to as I.A.U.) in the biography of Ibn Buṭlān, which he includes in his *ʿUyūn al-Anbāʾ*, a biographical encyclopaedia of physicians, originally composed around 1242. Ibn Buṭlān was not a professional astronomer or astrologer but, in the tradition of Hippocrates and Galen, he, like other physicians of his time, was concerned with the presumed connection between cosmic and telluric happenings and diseases and other natural catastrophes affecting the life and health of man. The report[18] reads as follows:

0028–0836/78/0273—0728$01.00

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"I (I.A.U.) have copied the following from an account in his (Ibn Buṭlān's) own hand. He says: 'One of the well-known epidemics of our own time is that which occurred when the spectacular (*athārī*) star (*kawkab*) appeared in Gemini in the year 446 H. (12 April 1054–1 April 1055 AD). In the autumn of that year fourteen thousand people were buried in [the cemetery of] the Church of [St] Luke, after all the [other] cemeteries in Constantinople had been filled. Then, in midsummer of the year forty-seven (447 H.: 2 April 1055–20 March 1056) the Nile was low and most people in Fusṭāṭ (old Cairo) and all the strangers died, except those whom Allāh willed to live. The epidemic spread to Iraq and affected most of the population, and the land was laid waste in the wake of contending troops, and this continued until the year 454 H. (15 Jan. 1062–3 Jan. 1063). In most countries people fell ill with black-bile ulcers and swelling of the spleen. The [usual] arrangement of the rise and fall of the fevers (the periodicity of the fever) was altered and the order of the crises was upset, so that the [rules of the] science of prognosis had to be changed.'

And he (Ibn Buṭlān) continues: 'As this spectacular (*athārī*) star (*kawkab*) appeared in the sign of Gemini which is the ascendant of Egypt, it caused the epidemic to break out in Fusṭāṭ when the Nile was low, at the time of its appearance in the year 445 H. (23 April 1053–11 April 1054). Thus Ptolemy's prediction came true: 'Woe to the people of Egypt when one of the comets (*al-dhwā'ib*) appears threateningly (*wa injahama*) in Gemini!'. Then, when Saturn descended into the sign of Cancer, the destruction of Iraq, Mosul and the Jazīra was completed; Diyār Bakr, Rabīᶜa, Muḍar, Fārs and Kirmān, the lands of the Maghrib, Yemen, Fusṭāṭ and Damascus/Syria were upset; the affairs of the kings of the world were disturbed; and wars, famine and epidemics abounded. And this confirmed the wisdom of Ptolemy in saying: 'When Saturn and Mars are in conjunction in the sign of Cancer, the world will be shaken.'"

The meaning of the adjective '*athārī*' is not entirely clear. A related word is used as a noun ('*athar*') in describing the supernova of 1006: Goldstein[17] translates '*athar*' as 'spectacle'. We think the sense of the word implies a novel astronomical or meteorological phenomenon most generally characterised by its transient, explosive or spectacular appearance. Apparent star, phenomenon or spectacle might be equally viable translations. The Arabic translations of Aristotle's 'Meteorology' usually apply the same expression to refer to new or apparent astronomical phenomena, without, however, making a clear distinction between rainbows, haloes, meteors, comets, novae and so on. Calling the object '*athārī* star' seems to reinforce the notion that it is both a new phenomenon *and* star-like (as opposed to, say cometary).

Before continuing with the astronomical significance of this report, the question of the date of the appearance of the '*athārī* star' must be considered. In the first paragraph of the report, where the star is mentioned in association with an epidemic in Constantinople, it is said to have appeared in the year 446 H. In the second paragraph it is associated with an epidemic in Fusṭāṭ (old Cairo) and its date is given as 445 H. As the reference is obviously to the same star in both cases, one of the dates—presumably the latter—must be erroneous, although the second reference to the year appears in all the manuscripts.[19]

The problems seems, however, to be solved when we compare the above report with another description by Ibn Butlān of events in Egypt at this time. This is to be found in a different part of the same work by I.A.U.—in his biography of Ibn Riḍwān, an Egyptian Muslim physician and astrologer who was a contemporary of Ibn Buṭlān. It runs as follows:

"At the time when Ibn Riḍwān was living there (in the Qasr ash-Shamᶜ quarter of old Cairo), a great famine occurred in Egypt and there was a terrible exodus, as a result of which most of the inhabitants perished. I quote a handwritten report of al-Mukhtar ibn al-Hasan ibn Buṭlān: 'The famine began in Egypt in the year 445 H. (1053–1054 AD). In the following year the Nile was low and the famine increased and was followed by a great epidemic, and it grew worse in the year 447 H.'"[20]

The star is not mentioned in this passage, which begins with the report of a famine in Egypt in 445 H. As regards "the following year", that is, 446 H., the events described are all found in the second paragraph of the first report, where, however, they are said to have occurred in Egypt in the year 445 H. I.A.U. himself thus seems to be responsible for copying the wrong date here. Apparently he overlooked Ibn Buṭlān's statement that this was the year preceding that in which the epidemic broke out in Egypt. The correct date of this event and, correspondingly, of the sighting of the star, must therefore be 446 H. (12 April 1054–1 April 1055 AD). (The possibility of a transcription error is reinforced by the appearance of a similar error in a Japanese record of the event. It was reported to have occurred in the "4th" rather than the "5th" month of the "Tenki reign period" (which includes 4 July). Both Duyvendak[7] and Clark and Stephenson[1] take this simply to be an error.)

The two reports seem to have been taken from different works by Ibn Buṭlān. In the first, which forms part of an astrological polemic, Ibn Buṭlān omits to mention those catastrophes which preceded the appearance of the star: perhaps because this would not accord with Ptolemy's prediction. Yet, he ignores the fact that Ptolemy predicted the advent of a comet, rather than a star. As regards Ptolemy's second prediction, it is interesting to note that in 1062 there indeed occurred a conjunction of Saturn with Mars "in the sign of Cancer". Ibn Buṭlān might either have observed or computed this result, so that it does not necessarily support his observational reliability. In any case he fails to mention the fact that it did not precede the series of catastrophes, as Ptolemy had forecasted, but occurred in 1062 AD, the very year in which, as he himself says, the catastrophes came to an end.

As regards the source and context of the predictions attributed to Ptolemy, we have been unable to locate them in his *Almagest*, or in his astrological work, the *Tetrabiblos*. Nor are they to be found in the *Centiloquium*, a collection of astrological aphorisms wrongly ascribed to him. It is possible that they were taken from some commentary on one of the last two works, such as that of Ibn Riḍwān on the *Tetrabiblos*.

From the astronomical point of view, the report, as corrected, suggests the following interpretation. At some time between 12 April 1054 and 1 April 1055, "a spectacular star appeared in Gemini". If Ibn Buṭlān were using an astrological convention, the position of the star could have been given with respect to the location of the sun within the zodiac. Because of the precession of the equinoxes, the Crab nebula, in the presently defined constellation Taurus (but near the edge of the neighbouring Gemini), would be said to have "appeared in Gemini" around the year 1000. In fact, precession puts the Crab nebula very near the centre of the "sign of Gemini" at that time. Alternatively, if the constellation of Gemini were taken to begin 60° of longitude west of the "first point of Aries", then the supernova could have been said to appear in (the constellation of) Gemini. Ibn Buṭlān seems to imply that this event caused the epidemic in the following autumn, appearing when the Nile was low. This places the event in the summer of 1054—consistent with the Chinese record of its first appearance on 4 July 1054.

This sighting of the star by itself adds nothing new to our knowledge of the position, brightness, time variability, or other astronomical information which we would like to know about the supernova explosion of 1054. However, it suggests that medical writings from the medieval islamic world, with their astrological (and astronomical) association, might provide a valuable new source for ancient astronomy. In any case, if the present report hardly justifies the claims of astrology, it restores our confidence in medieval Arab astronomy.

We thank P. Morrison, B. Marsden, B. Goldstein and D. Pingree for helpful discussions, and A. I. Sabra for his clarifications of the translation and for help in interpreting the Arabic text.

KENNETH BRECHER

Department of Physics,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

ELINOR LIEBER
ALFRED E. LIEBER

13 Balfour Street,
Jerusalem, Israel

Received 5 April; accepted 17 April 1978.

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Primitive atmosphere and implications for the formation of channels on Mars

THE channels on Mars[1,2] suggest that a flowing fluid has been present on the surface of the planet. It seems natural to assume that this fluid was water. The major difficulty, however, is that water freezes in climatic conditions like those now[3,4] on Mars. It has been suggested[5–7] that primitive Mars had a reducing atmosphere, composed mainly of methane. Such an atmosphere, as we show here, could be polymerised by solar ultraviolet radiation to produce higher hydrocarbons. These compounds are low viscosity liquids at today's temperature on Mars, and could contribute to the formation of channels.

We adopt a simple model of the early martian atmosphere similar to Sagan's[6] and Pollack's[7], except for ammonia. The total pressure equals 100 mbar, with CH_4, H_2 and N_2 as major components in proportions 60:6:1 by volume. The thermal structure of this atmosphere is similar to that given by Yung *et al.*[8] The amount of water vapour is then determined by assuming 50% relative humidity. The physical parameters of our model closely resemble those on Mars today. Ammonia was included in Sagan's model mainly to provide a greenhouse effect, but we omit for simplicity. Ultraviolet light dissociates CH_4 directly or indirectly by

$$\begin{aligned} CH_4 + h\nu &\rightarrow CH_2 + H_2 \\ &\rightarrow CH + H_2 + H \end{aligned} \qquad \lambda \leq 1{,}600\ \text{Å} \qquad (1)$$

and

$$CH_4 + OH \rightarrow CH_3 + H_2O \qquad (2)$$

where the radical OH is derived from

$$H_2O + h\nu \rightarrow H + OH \qquad \lambda \leq 2{,}400\ \text{Å} \qquad (3)$$

Reaction (1) occurs primarily in the upper armosphere, followed by reactions similar to those in the jovian atmosphere[9]. Reaction (2) provides the driving force for photochemistry in the lower atmosphere. Every dissociation of a water molecule, equation (3) brings about the dissociation of a hydrocarbon molecule.

The subsequent reactions leading to the formation of higher hydrocarbons are described in Table 1 and can be summarised by

$$nCH_4 + (H_2O) \rightarrow C_nH_{2n+2} + 2(n-1)H + (H_2O) \qquad (4)$$

where H_2O plays the part of a catalyst. The system is analogous to that studied by Lasaga, Holland and Dwyer[10] in connection with a primordial oil slick on Earth but differs in one major aspect. We use the photolysis of water, which is much larger than that of methane to drive the alkane photochemistry in the lower atmosphere, where associative reactions of alkyl radicals are efficient. The results of a steady state calculation are shown in Fig. 1. Comparison with Table 2 shows that the alkanes beyond C_7H_{16} would condense into a liquid. Using the production rates of alkanes in our model, we estimate that a major fraction of the atmosphere could be polymerised in less than 10 Myr giving rise to a layer of liquid alkanes as thick as 1 m if uniformly spread over the planet's surface. If we had started with 1 bar of methane, the amount of liquid would be 10 m.

Escape of hydrogen plays a crucial part in the evolution of a methane dominated atmosphere. With equation (1) as a high altitude source of hydrogen atoms the escape rate would be of order 10^{10} atoms $cm^{-2}s^{-1}$, or about 100 times higher than the present escape rate[11]. The loss of hydrogen leaves a surplus of carbon atoms and the system must evolve towards a state with lower H:C ratio by converting methane into higher hydrocarbons. It is now clear that given an initial methane atmosphere on a small planet like Mars, hydrogen escape and polymerisation are inevitable. Upon completion of polymerisation,

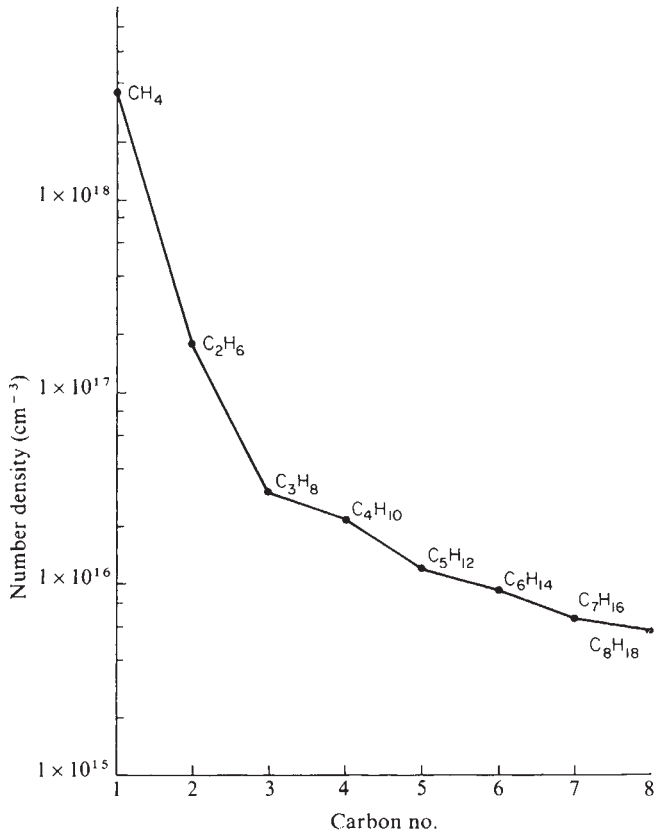

Fig. 1 Number densities of the lighter alkanes in the lower atmosphere of Mars. The total atmospheric pressure equals 100 mbar. The surface temperature is 220 K, and the adiabatic lapse rate = 1.9 K km^{-1}.

0028–0836/78/0273—0730$01.00

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Table 1 Major reactions in the early martian atmosphere

$H_2O + h\nu \rightarrow H + OH$	J	Ref. 8
$CH_4 + h\nu \rightarrow CH_2 + H_2$	J	9
$\rightarrow CH + H_2 + H$		
$C_2H_6 + h\nu \rightarrow C_2H_2 + 2H_2$	J	9,16
$\rightarrow C_2H_4 + 2H$		
$C_nH_{2n+2} + h\nu \rightarrow$ Products	J	18
$CH_2 + H_2 \rightarrow CH_3 + H$	$k = 5.0 \times 10^{-14}$	9
$OH + H_2 \rightarrow H_2O + H$	$k = 3.6 \times 10^{-11} \exp(-2590/T)$	17
$H + H \underset{M}{\rightarrow} H_2$	$k = 1.0 \times 10^{-32}$M	9
$C_2H_2 + H \underset{M}{\rightarrow} C_2H_3$	$k = 5.0 \times 10^{-29} \exp(-1213/T)$M	19
$C_2H_3 + H \rightarrow C_2H_2 + H_2$	$k = 1.5 \times 10^{-11}$	20
$CH_4 + OH \rightarrow CH_3 + H_2O$	$k = 2.4 \times 10^{-12} \exp(-1710/T)$	17
$CH_3 + H \underset{M}{\rightarrow} CH_4$	$k = 1.0 \times 10^{-10}$	21
$CH_3 + CH_3 \underset{M}{\rightarrow} C_2H_6$	$k = 4.0 \times 10^{-11}$	9,22
$C_2H_6 + OH \rightarrow C_2H_5 + H_2O$	$k = 1.9 \times 10^{-11} \exp(-1230/T)$	17
$C_2H_5 + CH_3 \underset{M}{\rightarrow} C_3H_8$	$k \approx 4.0 \times 10^{-11}$	9,22
$C_2H_5 + C_2H_5 \underset{M}{\rightarrow} C_4H_{10}$	$k \approx 4.0 \times 10^{-11}$	9,22
$C_nH_{2n+2} + OH \rightarrow C_nH_{2n+1} + H_2O$	$k = (2n+2) \times 10^{-12} \exp(-820/T)(n \geqslant 3)$	17
$C_nH_{2n+1} + C_mH_{2m+1} \underset{M}{\rightarrow} C_{m+n}H_{2(m+n)+2}$	$k \approx 4.0 \times 10^{-11}$	9,22

The units for photolysis rates (J) and two body reaction rates (k) are s^{-1} and $cm^3\,s^{-1}$ respectively. M denotes number density of a third body (in units of cm^{-3}). Where M does not explicitly appear in a three body reaction rate k is taken to be the two body rate at high pressure.

the initial resource of hydrogen by equation (4) is exhausted, and further escape of hydrogen must be supplied from water, with production of oxygen. Oxygen will now oxidise the alkyl radicals by reactions such as:

$$CH_3 + O \rightarrow CH_2O + H \quad (5)$$

followed by

$$CH_2O + h\nu \rightarrow CO + H_2 \quad \rightarrow HCO + H \quad (6)$$

$$HCO + h\nu \rightarrow CO + H \quad (7)$$

The appearance of CO initiates a catalytic cycle that competes with equation (2) for OH:

$$CO + OH \rightarrow CO_2 + H \quad (8)$$

$$CO_2 + h\nu \rightarrow CO + O \quad (9)$$

While equations (2) and (3) form a closed loop for H_2O, equations (3), (8) and (9) result in releasing more oxygen atoms for reactions such as equation (5). Thus, all the alkanes could be destroyed in perhaps as short a time as it had taken to make them.

The channels on Mars are a complex phenomenon and probably no single explanation would suffice. Our photochemical model provides a modest amount of fluid (1–10 m) for a brief interval of time (10–100 Myr). The existence of liquid alkanes complements rather than competes with other mechanisms for the formation of channels. A preliminary investigation suggests that the alkanes do not contribute significantly to greenhouse effects but their associated products such as C_4H_6O and C_3H_7N may. Also compounds like CH_3OH that can depress the freezing point of water may be present. We shall include these compounds in an extended study.

Two crucial aspects of this theory can be tested by suitable measurements. The assumption of an early massive reducing atmosphere is based on Holland's[12] work on the evoluion of the Earth's atmosphere. There is more evidence from an analysis of D/H ratio[13] to support this hypothesis. A similar measurement of D/H ratio of water on Mars would yield

Table 2 Properties of simple alkanes

		C_5H_{12}	C_6H_{14}	C_7H_{16}	C_8H_{18}	H_2O
Freezing point (K)		143	178	182	216	273
Viscosity (cP)		0.29	0.41	0.52	0.71	1.8
Heat of fusion (cal g^{-1})		28	36	34	43	80
Heat of vaporisation (cal g^{-1})		92	89	89	81	540
Number density of saturated vapour (cm^{-3})	160 K	1.8×10^{15}	1.2×10^{14}	6.9×10^{12}	1.2×10^{12}	3.2×10^{10}
	180 K	1.6×10^{16}	1.5×10^{15}	1.4×10^{14}	2.7×10^{13}	2.1×10^{12}
	200 K	9.2×10^{16}	1.2×10^{16}	1.5×10^{15}	3.1×10^{14}	5.8×10^{13}
	220 K	3.8×10^{18}	6.0×10^{16}	1.1×10^{16}	2.4×10^{15}	8.7×10^{14}

Water is included for comparison. Viscosity is taken at 0°C, but it is not a strong function of temperature. Data come from ref. 23.

valuable information on the amount of hydrogen that has escaped. The evolution of a methane atmosphere can best be tested on Titan[14], whose atmosphere bears striking similarities to our model of the primitive martian atmosphere, especially in view of a measurement[15] of a surface temperature in excess of 200 K. The detection of higher alkanes or carbon monoxide on the satellite would be expected on the basis of our photochemical model.

Y.L.Y. thanks Professor M. B. McElroy for valuable insights on atmospheric evolution, hydrogen escape and isotopic ratio and J. B. Pollack and O. B. Toon for discussion of present and past climates on Mars. This research was supported by Ames Research Center under NASA contract NSG-2283. J.P.P. acknowledges support by NASA grant NSG-5163 to Columbia.

Y. L. YUNG

California Institute of Technology, Pasadena, California 91125

J. P. PINTO

Goddard Institute for Space Studies, New York, New York 10025

Received 6 February; accepted 3 May 1978.

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Meteor radar rates, geomagnetic activity and solar wind sector structure

THERE is evidence which indicates[1–4] that the occurrence rate of meteor radar echoes varies inversely with solar cycle activity, this phenomenon can be explained[5–6] by a long-term variation in the atmospheric density gradient at the meteor ablation level. Inferred changes in the atmospheric properties are attributed to a long-term variation in the solar EUV radiation. Although the long-term, solar cycle changes in radar-determined meteor rates show an inverse correlation with sunspot numbers and other measures of solar activity, such correspondence is not readily evident in the day-to-day meteor radar data. One reason for this is the inability of most solar activity indices to measure the relevant solar parameters involved in short-term solar–terrestrial effects. The study of solar-influenced meteor rate changes on a time scale of days has, therefore, not received adequate attention. Evidence for a short-term dependence of meteor radar rates on geomagnetic activity and solar corpuscular radiation is presented here.

This investigation is based on the long series of radar observations of meteors made at the Onsala Space Observatory in Sweden. These were carried out during pre-selected time periods in August and September, with occasional runs in October and November. Our radar equipment operated at 32.6 MHz and recorded meteor echoes of duration >0.02 s corresponding to visual meteors brighter than about fifth zenithal magnitude. The experimental technique has been described elsewhere[7]. Here we analyse night-time recordings (2100–0300 UT) made between 1953 and 1974 and daytime recordings (0700–1300 UT) made between 1960 and 1974. The original echo rate data are available on a 1-h basis. In the present study 6-h averaged total meteor rates for a night or day-period are analysed.

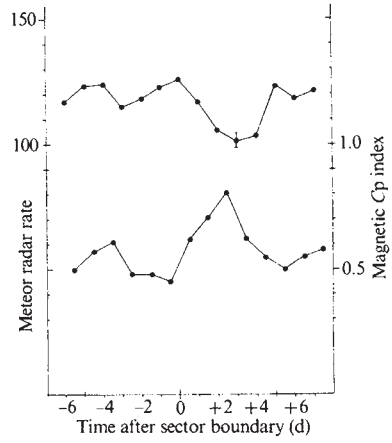

Fig. 1 Variation of meteor radar rates and geomagnetic activity with time measured from the passage of a solar magnetic sector boundary past the Earth. Superimposed-epoch analysis of all meteor radar data August–November 1953–74. Top curve: radar-determined meteor hourly rate (left scale); lower curve: geomagnetic C_p index for days of radar recordings (right scale). Radar rates are referred to the nearest 00 UT, geomagnetic activity to the nearest 12 UT. Sector boundaries as given by Svalgaard are referred to the nearest 00 UT. Each datum point in Fig. 1 represents the mean of 35–49 individual measurements of the radar rate. The s.e.m. varies from 4.4 to 8.0 units. A vertical bar indicates the standard error of the mean for epoch day +3.

The short-term response of meteor radar echo rates to geomagnetic activity and solar corpuscular radiation was investigated with the technique of superimposed epochs. This technique has been used for many years in the study of solar–terrestrial relationships, and recently several meteorological and geophysical phenomena have been correlated with the solar wind sector structure[8–10]. In our study, we therefore investigated whether meteor radar echo rates and geomagnetic activity were influenced by the passage time of the solar wind sector boundary. The atlas of well-defined sector boundaries prepared by Svalgaard[11] was used to define the key day (zero day) in the superimposed epoch analysis. An advantage of the superimposed epoch technique is that variations unrelated to the sector structure—such as meteor rate changes of an astronomical nature—will tend to cancel out in the superimpositioning process.

The results of our analysis are shown in Figs 1–4. In each case, the top curve depicts the variation of the mean hourly rate of meteor radar echoes as the solar sector structure is carried past the Earth by the solar wind. The lower curve shows the corresponding variation of the geomagnetic C_p index.

An analysis of all available meteor data 1953–74 is given in Fig. 1. The following features are evident: an increase in geomagnetic activity begins after sector boundary passage, with a maximum occurring about 2.5 d later. A broad minimum in meteor radar echo rates begins after passage of the sector boundary and is most pronounced near epoch day +3. A secondary maximum in geomagnetic activity and a secondary minimum in radar rates are seen a few days before sector boundary passage. Although the latter features are not very prominent in Fig. 1 they are also observed when the data are grouped in other ways (Figs 2–4).

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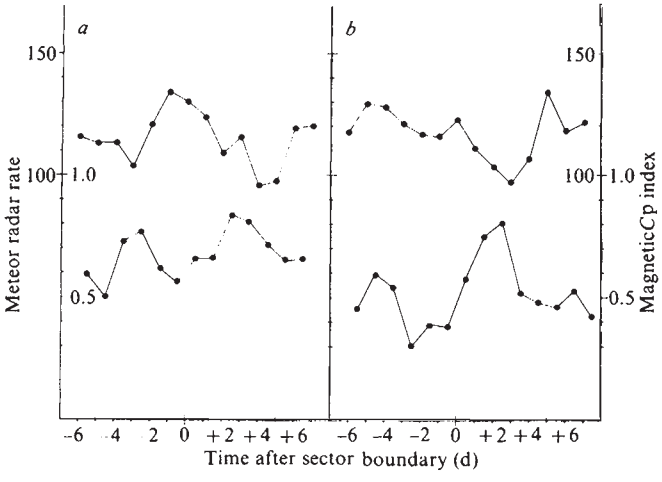
The maximum of the Perseid meteor shower, which occurs around 12 August, is included in our August sample. The position of the Perseid maximum will tend to occur at random phases within the sector structure. Its influence will, therefore, be averaged out in a large data sample. This point was checked in several ways. In one superimposed epoch study August and September meteor rates were analysed separately. The peaks and valleys in Fig. 1 were evident in both samples. They were, however, most pronounced during September, when no intense meteor shower was present. In another study, days of peak Perseid activity were eliminated by sorting out solar longitudes 138.0–140.9°. No significant change in the diagrams occurred. Note that the activity of a meteor stream is mainly evident as an increase in the number of long duration radar echoes. A separate superimposed epoch analysis was, therefore, carried out, in which all long duration radar echoes were eliminated. Results similar to those of Fig. 1 were obtained. We conclude therefore that effects observed in Fig. 1 are independent of meteor shower activity.

The magnitude of the radar rate depression in Fig. 1 is substantial. At epoch day +3 the minimum is approximately 20% of the average value, or about 25 units. The standard error of the mean is 6.3 units as indicated by the error bar in Fig. 1. It is, however, difficult to assess the significance of the peaks and valleys in Fig. 1 by ordinary statistical tests, primarily because recordings from one day to the next are not necessarily independent of one another. The significance and reproducibility of the results shown in Fig. 1 was investigated instead by dividing the data sample into two parts and carrying out the same superimposed epoch analysis separately on each part.

Figure 2 shows the results of a separate analysis of radar echo rates recorded in 1953–63 and 1964–74. The previously observed effects are also noticeable in the smaller data samples of solar cycles 19 and 20. Figure 2 suggests that the dependence of radar echo rates and geomagnetic activity on solar wind sector structure is strongest in the 1964–74 data. This may indicate that the sector boundary crossings are less well defined in the pre-satellite era, or that the geomagnetic response to the solar wind has changed over the period covered by our study.

Figure 3 compares meteor rates for night- and day-recordings in the 1960–74 period. Both data sets show the effects previously depicted in Fig. 1. The echo rate minimum on epoch day +3 is slightly more pronounced in the night-time data. An enhanced radar rate response to sector structure during night-time recordings was also evident when the data were grouped in other ways. This is not surprising, as solar EUV radiation is

Fig. 2 Variation of meteor radar rates and geomagnetic activity with time measured from the passage of a solar magnetic sector boundary. The analysis is the same as that in Fig. 1, except that data from solar cycles 19 and 20 have been treated separately. *a*, 1953–63; *b*, 1964–74.



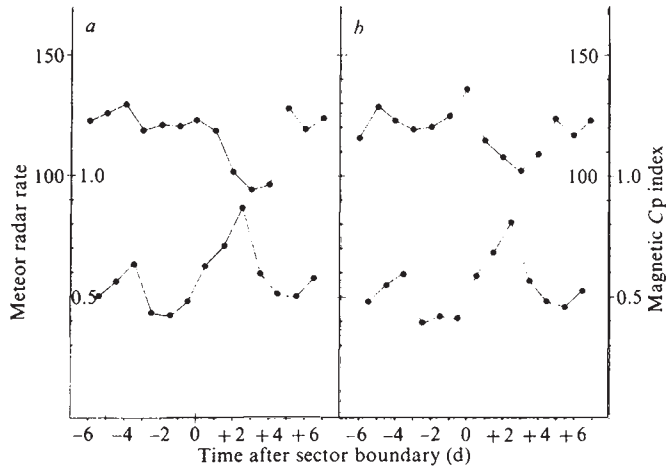


Fig. 3 Variation of meteor radar rates and geomagnetic activity with time measured from the passage of a solar magnetic sector boundary. *a*, Night-time recordings 1960–74; *b*, day-time recordings 1960–74.

the dominating heating agency in the day-time upper atmosphere.

In Fig. 4, the polarity of the interplanetary magnetic field is taken into consideration and away/towards and towards/away sector boundaries are analysed separately. The already established behaviour of meteor radar rates and geomagnetic activity for the first few days after a sector boundary crossing is evident. For days before the boundary crossing, however, we observed a striking difference between the two types of transitions. Data referring to the transition from away to towards polarity of the interplanetary magnetic field is shown in Fig. 4*a*. Note the increased geomagnetic activity on epoch days −3 and −4 and the minimum in meteor radar rates on epoch days −2 and −3. In contrast to this behaviour, Fig. 4*b* shows exceptionally high echo rates on days −2 and −3 and very low geomagnetic activity for several days before the boundary crossing.

A rapid increase in geomagnetic activity near and after the sector boundary passage has been previously reported. In an analysis of the C_p index Shapiro[13] found that the most significant features were a minimum in geomagnetic activity preceding passage of a towards/away sector and a maximum at the beginning of an away (+) sector; these features are clearly shown in Fig. 4.

A striking feature of the present analysis is the large response of meteor rates to variations in geomagnetic activity and/or to sector structure. There is no obvious way in which the incident meteoroid population can adjust to geomagnetic activity or to the solar wind sector structure. Hence, we conclude that the meteor rate variation is an apparent effect ascribable to transient changes in the Earth's atmosphere at the 90–110 km level. The fact that radar-determined meteor rates vary with geomagnetic activity as the solar sector structure is swept past the Earth indicates that we are observing a solar wind induced 'geomagnetic' heating of the upper atmosphere.

Atmospheric perturbations of geomagnetic origin have been inferred from satellite drag data[14–16]. These observations show transient increases in air density at altitudes above 150 km a few hours after a geomagnetic disturbance. Orbital drag data from low altitude satellites in the region 140–170 km have revealed density variations of a geomagnetic nature in phase with those at higher altitudes. Ching[17] reports density increases of up to 25% at 170 km after geomagnetic disturbances of moderate strength. During a geomagnetic storm a density enhancement at 140 km of about 35% was observed. Such a substantial increase indicates that the source of geomagnetic heating was at very low altitudes.

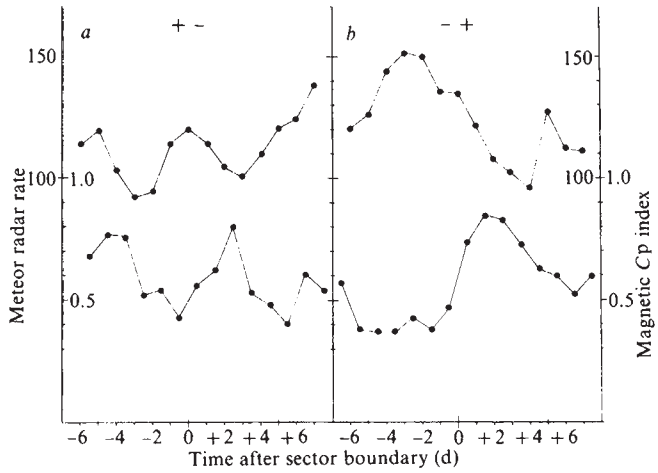


Fig. 4 Average response of meteor radar rates and geomagnetic activity to the solar wind magnetic sector structure 1953–74. Separate superimposed epoch analysis for: *a*, away/towards sectors; and *b*, towards/away sectors.

The detection capability of a meteor radar varies with the atmospheric density gradient[6]. Other parameters being equal, recorded echo rates N are given by an equation $N = \text{constant} \times (d\rho/dh)^p$, where $(d\rho/dh)$ is the mean density gradient in the meteor zone. The exponent p depends on the mass index s of the meteor population and on the degree of aspect sensitivity of the ionised trails. From our visual meteor observations we find $s = 2.7$. We also note a substantial loss of radar aspect sensitivity for meteors in the range 0–4 mag. Neglecting the effect of aspect sensitivity we deduce $p = s - 1 = 1.7$. The meteor radar rate data shown in Figs 1–4 indicates a 20–25% depression in radar-determined meteor rates at epoch day +3. This corresponds to a 10–15% reduction in the mean density gradient in the meteor zone (90–110 km) following a geomagnetic disturbance.

Based on a study of a limited number of radar–visual meteor starting heights we find that air density at 110 km has remained constant during the disturbance. It follows that a density increase has occurred at some lower level in the meteor zone. An antiphase relationship thus exists between the inferred density changes at satellite and meteor altitudes[2]. This is what one would expect if we were observing an atmospheric heat source located in the 110–140-km region.

The mechanism by which the appropriate atmospheric layers are heated when the Earth's magnetic field varies is not well understood. Joule heating has been proposed as the process responsible for the temperature and density fluctuations in the neutral atmosphere[18]. The occurrence of simultaneous changes in composition of the atmosphere complicates the picture.

Recent studies[19–20] have demonstrated the enhanced geomagnetic effectiveness of a southwards component in the interplanetary magnetic field. It follows that a different geomagnetic response to solar wind sectors depending on the type of polarity change is possible. This effect, which has been previously observed[13,21,22], is very noticeable in our data (Fig. 4). The sector structure observed at Earth during August–October is basically of the two-sector type[23]. We may, therefore, tentatively connect the curves in Fig. 4 to get an approximate 27-d picture of the variation of radar rates and geomagnetic activity over a complete solar rotation. The reduction in geomagnetic activity and increase in radar rates in the trailing part of a towards (−) sector is particularly noticeable.

Statistical investigations show that the high speed particles in the solar wind tend to occur at fixed positions within the sector structure[24]. In giving a physical interpretation to the correlations between meteor radar rates, magnetic disturbances and the sector structure, one must further study the interaction between the high speed streams in the solar wind and the Earth's magnetosphere. It is hoped that future coordinated studies of meteor radar echo rates, geomagnetic activity, and solar wind parameters will help us to understand some of the complicated interactions between the Sun and the Earth's upper atmosphere. Statistical studies of this nature—involving various layers of the upper atmosphere—may also lead to a better understanding of the physical mechanisms involved in solar–terrestrial relations, including Sun–weather influences.

This study was supported by grants from the Swedish Natural SRC, The Swedish Board for Space Activities, Knut och Alice Wallenbergs Stiftelse and Riksbankens Jubileumsfond. I thank L. Andreásson and T. Wiesel for technical assistance and Dr P. Janecek for computer programming.

B. A. LINDBLAD

Lund Observatory,
S 222 24, Lund, Sweden

Received 4 January; accepted 19 April 1978.

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Kirkwood Gaps and stability of conservative periodic systems

KIRKWOOD GAPS[1] are gaps in the distribution of mean motion of asteroids at the multiples 2/1, 5/2, 3/1 and so on, of Jupiter's mean motion; but at the value 3/2, there is actually a concentration. Figure 1 shows the distribution of the best-determined orbits in the (semi-major axis, eccentricity)- or (a, e)-plane around the 2/1 resonance (the Hecuba Gap) and the 3/2 resonance (the Hilda Group). The sharp contrast between the two cases, especially in the eccentricity range $0.15 < e < 0.25$, demonstrates the problem, and I show here that the Hilda region is stable in a well-defined sense, and the Hecuba region is unstable.

My solution does not consider non-gravitational agents such as mutual collisions or resisting media. I start with a purely gravitational model of the simplest kind—the planar, circular model of three bodies. Schubart[2] showed that if we average out short-period variations over a period of approximate recurrence, then the model is further simplified to a single-infinity (parameter U) of conservative systems of one degree of freedom (canonical variables S, σ, Hamiltonian F). A constant U corresponds to a fixed curve in the (a, e)-plane:

$$U \equiv \sqrt{a} + \alpha\sqrt{a}[1 - (1 - e^2)^{1/2}] = \text{constant} \qquad (1)$$

($\alpha = 1$ for Hecuba, 2 for Hilda). The meanings of S and σ, in terms of a, e, the perihelion longitude ω' and the mean longi-

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tudes λ, λ' are

$$S=\sqrt{a}[1-(1-e^2)^{1/2}], \qquad \sigma=(\alpha+1)\lambda'-\alpha\lambda-\omega' \tag{2}$$

The system being conservative, the orbits are the curves

$$F\equiv F(S,\sigma;U)=\text{constant} \tag{3}$$

on a given U-plane. It is convenient to draw these closed orbits using (e, σ) as polar coordinates, and I shall refer to them as a Schubart diagram. Regarding the orbits as contour lines of a country, a typical Schubart diagram consists of a crescent-shaped valley, enclosing a hill and surrounded by high-rising mountains on all sides. The orbits in the valley are the 'librators'; they correspond to slow oscillations of large amplitudes across the exact resonance along the curve (1) in the (a, e)-plane. The orbits on the hill and the mountains are 'circulators'; they correspond respectively to fast oscillations of small amplitudes in the bottom and top part of the curve, away from the resonance point.

This very simple system is, in one respect, more complete than those of Poincaré[3] and E. W. Brown[4], here the finite eccentricity of the asteroid is taken in full and not treated as a small parameter. The Schubart diagrams for Hecuba and Hilda are rather alike in general appearance[2] (Figs 1–10), and this has led to the view[5] that Jupiter's eccentricity must be invoked to account for the Hecuba–Hilda difference. I however believe that it is entirely a question of stability of the orbits—a question that has not previously been examined.

Poincaré's[6] theory of characteristic exponents implies that a conservative periodic system of one degree of freedom is always unstable. A short geometrical proof of this theorem will clarify that the 'stability' it refers to is not the kind I am interested in. The orbits for such a system are a set of non-intersecting, closed curves in the 2-dimensional phase space, each determined by any of its points. A small perturbation means the system is moved to a point not on the original curve. The Classical Injunction (CI) then says 'Let the perturbed system evolve under the given Hamiltonian'. The perturbed system then moves along the orbit as determined by its initial position. This orbit will in general have a different period from the original one. Hence the distance between the perturbed and the original systems must in general increase with time, and that means instability in the sense Poincaré uses the word.

I would like to be able to say that the Hecuba–Hilda difference is due to the Hilda librators being stable and the Hecuba librators being unstable. Therefore, my definition is that an orbit is stable if, given a small perturbation, the system will remain close to the orbit. So I am interested in the eventual deviation from an orbit, whereas Poincaré[7] was concerned with deviation from a certain point on an orbit representing the original, unperturbed system. The difference becomes apparent when we note that every system obeying the CI must be always stable on my definition. My criterion of stability must be such that the stability depends entirely on the individual orbit concerned so that the verdict on a given orbit is never known in advance. In trying to prove that an observed 600-year periodicity in the residuals of Comet Halley is a period inherent in the system Sun–Jupiter–Halley idealised to be periodic with a period of 154.2 yr, I succeeded in deriving a Hill's equation for the residual, that is a second-order differential equation involving no dependent variables other than the residual and with known, periodic functions as coefficients. The value of Hill's exponent c turned out to be close to the target value of 0.25 for most values of the initial longitude of Jupiter; but for some, it turned out to be imaginary. An imaginary c means the residual will grow indefinitely, that is, the system is unstable. At the time[8] I was not interested in the imaginary values, but I gradually realised that we can make a completely new and important use of Hill's equation[9] as the backbone of a theory of stability: 'a periodic system is said to be stable or unstable according to whether the solution to its associated Hill's equation is stable or unstable, that is according as the Hill's exponent c is real or imaginary' (criterion of stability).

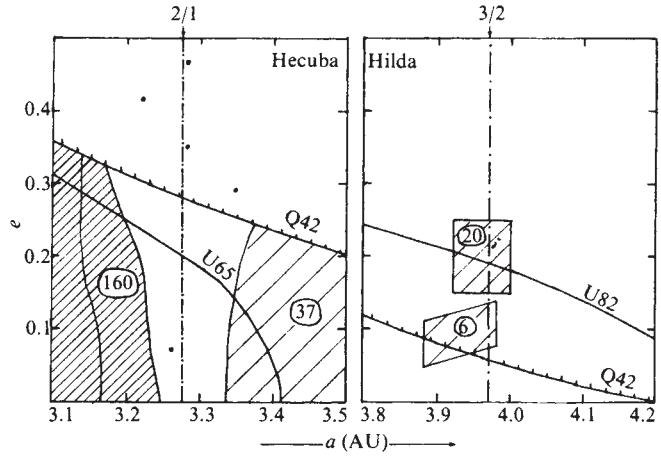

Fig. 1 The distribution of asteroids in the (a, e)-plane is vastly different in the 2/1 and 3/2 resonance regions. In the former, there is a near-void around the resonance, flanked on both sides by areas of high density (shown shaded, with number of objects in circles); in the latter, all known objects cluster about the resonance point. According to theory, an asteroid oscillates along curves like U65 ($\sqrt{a}[2-\sqrt{(1-e^2)}]=\sqrt{0.655}$) and U82 ($\sqrt{a}[3-2\sqrt{(1-e^2)}]=\sqrt{0.82}$) with amplitudes and speeds depending on its location on the curve and on another parameter σ. The oscillations across a resonance point are large and σ librates to prevent close approaches to Jupiter. The stability theory given here shows that these oscillations are unstable across the 2/1 resonance and stable across the 3/2 resonance, thus accounting for the observed difference. Q42 is the line $a(1+e)=4.2$ AU, above which a non-librator cannot survive long the Jovian sweep. This largely explains why there are no asteroids in the 3/2 resonance region apart from the Hilda librators.

A principle is needed by which a Hill's equation for any given canonical equations of one degree of freedom can be written. This principle is the direct opposite of the CI, because the CI always prescribes the results in advance. To discover the alternative principle, we try to derive a second-order differential equation for the variation under the CI. I use x, y for the canonical variables, ξ, η for their variations, F for the Hamiltonian, the suffix convention for partial derivatives, and numerical suffixes (1 for x, 2 for y) to indicate evaluation at (x, y) after differentiation. The basic equations are then

$$\frac{dx}{dt}=F_2, \qquad \frac{dy}{dt}=-F_1 \tag{4}$$

According to the CI, the second-order equation in ξ must be found as the difference between

$$\frac{d^2x}{dt^2}=F_{21}F_2-F_{22}F_1 \tag{5}$$

and the equation for $d^2(x+\xi)/dt^2$. The result is

$$\frac{d^2\xi}{dt^2}=\frac{dF_{21}}{dt}\xi+\frac{dF_{22}}{dt}\eta+(F_{12}F_{12}-F_{11}F_{22})\xi \tag{6}$$

This is not a Hill's equation, because of the term in η. It would be, if the last term alone were present in the RHS:

$$\frac{d^2\xi}{dt^2}=(F_{12}F_{12}-F_{11}F_{22})\xi \tag{7}$$

Equation (7) can be obtained by adopting the following definition

$$\frac{d^2\xi}{dt^2}=\lim_{h\to 0}\frac{1}{h}\left[\frac{d}{dt}\left(x+\xi+h\frac{d\xi}{dt}\right)-\frac{d}{dt}(x+\xi)\right] \tag{8}$$

if it is understood that we expand all the first derivatives according to the basic canonical equations (4), never using

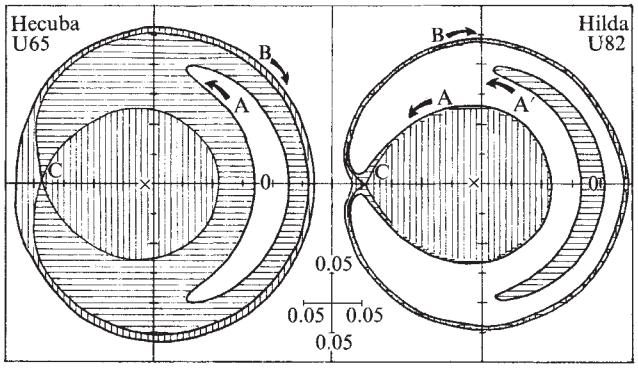

Fig. 2 Schubart diagrams for the lines U65 and U82 of Fig. 1. Polar coordinates (e, σ). On the basis of the theory of stability given here, we can mark out the stable regions (unshaded) and the unstable regions (shaded). In the Hilda diagram, the small crescent of instability is not effective enough to alter the generally stable character of the Hilda librators. In the Hecuba diagram, the crescent of stability does not imply the permanent existence of librators, because of approximations made in constructing the model. Hence the librators in the Hecuba region are generally unstable, and usually end up as stable circulators outside the orbit B, in a part of the (a, e)-plane, where large number of asteroids are found.

equation (5). This is equivalent to saying that, in the derivation, we regard the unperturbed coordinates (x, y) as having zero acceleration. The principle sought is therefore 'To be able to derive a Hill's equation from the system (4), we must refer the variations to an unaccelerated frame in the phase-space' (the enabling principle).

In contrast, our inability to derive a Hill's equation under the CI is because in that scheme, the variations are referred to the accelerated frame following the particle's natural motion, represented by equation (5).

The criterion of stability and the enabling principle are the core of the present theory of stability. In this theory, the stability of a conservative periodic system of 1 degree of freedom with Hamiltonian F depends entirely on the behaviour, over the given period, of the function

$$K(x, y) = F_{xx}F_{yy} - F_{xy}^2 \qquad (9)$$

or, more precisely, on its Fourier amplitudes. The evaluation of Hill's exponent c from these amplitudes should be made according to my earlier formulae[8] rather than using Hill's, because he dealt with a special case.

Further work is required on the following questions: one is uniqueness of Hill's equation; for example, $(\mathrm{d}F_{21}/\mathrm{d}t)\xi$ could be added to the RHS of equation (7) to get another Hill's equation. I can now rationalise the form of equation (7) by saying that it can be derived from a deeper principle. But by this rationalising, am I implicitly appealing to another, higher, principle? Another question is: if we use a different pair of canonical variables, will the results be different? In the application, I made calculations with both (S, σ) and $(\sqrt{2S}\cos\sigma, \sqrt{2S}\sin\sigma)$ for some orbits. While the final verdicts, when determinate, were never contradictory, in many instances where the (S, σ)-analysis finds instability, the other analysis fails to give a convergent $\Delta(0)$, a quantity necessary in the evaluation of c.

The foregoing theory enables me to mark out regions of stability and instability on the Schubart diagrams. The results for the two particular cases chosen are shown in Fig. 2. These results show that the Schubart diagrams for Hecuba and Hilda are altogether different in respect of stability. For the region of librators that is of the greatest concern, one is almost exactly the opposite of the other. For Hecuba, there is a crescent region of stability A at the bottom of the valley, while the greater part of the valley is unstable. There is nothing to prevent an unstable Hecuba librator drifting about and eventually ending up as a stable circulator with an eccentric orbit this side of the gap. (Many such objects are known). For Hilda, the stable region extends almost to the valley boundary delineated by the re-entrant orbit C. But there is an unexpected region of instability A' at the valley bottom. However, as an asteroid starting there can only wander off to the surrounding stable region, it does not affect our conclusion that the Hilda librators will permanently oscillate about the 3/2 resonance. The same conclusion was reached by Schubart[10] from numerical integration of the real objects.

The implication of the crescent of stability in the Hecuba diagram must be examined. It says that an orbit with $e = 0.2$, for example, will have an *a priori* probability of 0.4 of being stable. Why, then do we not see them—at least among the best determined orbits as there are a few such objects among the poorer orbits)[11]. We must remember that Schubart's[10] averaging process involves an approximation. It is supposed to be done over a period of recurrence, but as soon as there is a departure from the exact resonance, the system is no longer strictly recurrent. This means that the integral of equation (1) is only approximate, or the asteroid is constantly moving from one U-plane to another. Such movements can be shown to be most rapid for the Hecuba case. Other approximations used in the model, such as neglecting Jupiter's eccentricity, will contribute further to these extra-theoretical movements both within the same plane and across the planes. There is, then, always some finite probability of a stable orbit changing into an unstable one, or vice versa. The topology of the Hecuba diagram seems, however, to suggest that no dynamical equilibrium will result, and that the number of stable objects will decrease all the time.

The striking difference between Hecuba and Hilda, is thus accounted for by the present theory of stability.

T. KIANG

Dunsink Observatory,
Castleknock, Co. Dublin,
Ireland

Received 3 April; accepted 19 April 1978.

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Evidence for leptonic colour?

THE new Gargamelle data[1] on $\nu_\mu e$ scattering with 10 reported events of the type $\nu_\mu + e^- \rightarrow \nu_\mu + e^-$, where 1.7 ± 0.2 would be expected on the basis of the $SU(2) \times U(1)$ model (with $\sin^2\theta_W = 1/4$) taken at its face value, and ignoring problems connected with unconventional electron energy spectrum of the events, seem to suggest the existence of one (or more) new purely leptonic neutral currents, in addition to the familiar neutral current contained within $SU(2)_L \times U(1)$. To preserve[2] the excellent agreement of the standard $SU(2)_L \times U(1)$ model with the very accurate experimental data on semileptonic $\nu_\mu + N \rightarrow \nu_\mu + N$ and $\nu_\mu + N \rightarrow \nu + N_\mu + \pi$, the new neutral current phenomena must exhibit the minimal possible interference with the old current phenomena. The situation is reminiscent of colour currents for quarks which have a minimal interference

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with the flavour (SU(2)×U(1)) currents. By analogy with the three quark colours it seems that the concept[3] of a purely leptonic colour must be introduced, distinguishing between electronic (red) and muonic (yellow) varieties of leptons. The newly discovered leptons (τ^0, τ^-) may carry electronic or muonic colour or may define a third leptonic colour (blue). This colour quantum number is additional to leptonic flavour which distinguishes neutrinos from charged leptons.

Accepting flavour independence of the new neutral leptonic currents, there are just four possible currents (each representing the appropriate lepton number) which may be written as:

$$N_L^e = (\bar{\nu}_e\nu_e)_L + (\bar{e}e)_L;\ N_R^e = (\bar{e}e)_R,$$
$$N_L^\mu = (\bar{\nu}_\mu\nu_\mu)_L + (\bar{\mu}\mu)_L;\ N_R^\mu = (\bar{\mu}\mu)_R$$

Taken with the $SU(2)_L \times U(1)$-currents it seems that there is only one possible combination of the above which in a gauge theory context is free of anomalies. This is the unique current $1/2(N^e - N^\mu)$ where $N = N_L + N_R$ which could be part of the extended gauge-structure $SU(2)_L \times U(1)|_{\text{flavour}} \times U(1)_{\text{colour}}$ (one could introduce another anomaly-free current $(N_e + N_\mu - N_\zeta - N_{\zeta'})$ if there are further leptons ζ, ζ'. However such a current would lead to excessive enhancement for $\bar{\nu}_\mu e$ compared to its present experimental value). However, from its traceless form, it is clear that such a current is the third component of a SU(2)-colour triplet if electronic and muonic leptons form a colour doublet. We are thus motivated towards a gauge-group,

$$[SU(2)\times U(1)]_{\text{flavour}} \times SU(2)_{\text{leptonic colour}}$$

with

$$\begin{bmatrix} \nu_{eL} & \nu_{\mu L} \\ e_L^- & \mu_L^- \end{bmatrix}$$

forming $(2,2)_1$ and (e_R^-, μ_R^-) forming a $(1,2)_2$ of $SU(2)|_{\text{flavour}} \times SU(2)|_{\text{leptonic colour}}$. (The ambiguity in the choice between (e_R^-, μ_R^-) versus (μ_R^-, e_R^-) as $(1,2)_2$ doublets disappears within a left-right symmetric extension.) The subscripts on $(2,2)_1$ and $(1,2)_2$ designate the appropriate U(1) representation. Quarks are singlets of SU(2) leptonic colour.

Before constructing the appropriate gauge theory, note that the new current, taken in conjunction with the old SU(2)×U(1) predicts:

$$\frac{\sigma(\nu_\mu e \to \nu_\mu e)}{\sigma(\bar{\nu}_\mu e \to \bar{\nu}_\mu e)} = \frac{3 + 16\,(\sin^2\theta_W - \alpha^2)^2 - 12\,(\sin^2\theta_W - \alpha^2)}{1 + 16\,(\sin^2\theta_W - \alpha^2)^2 - 4\,(\sin^2\theta_W - \alpha^2)}$$

where

$$\alpha^2 = \frac{1}{2}\frac{g_1^2}{m_Y^2}\frac{m_Z^2}{g_0^2}$$

Here (g_1, m_Y) is the coupling constant and mass of the gauge particle Y associated with the new current and (g_0, m_Z) are the corresponding parameters for the old Z.

$$L = g_0 Z_0 (J_{3L} - \sin^2\theta_W J_{em}) + \tfrac{1}{2} g_1 Y (N_e - N_\mu),$$

with

$$J_{3L}^e = \tfrac{1}{2}(\bar{\nu}_e\nu_e - \bar{e}e)_L$$
$$J_{em}^e = -(\bar{e}e)_{L+R}$$

and

$$N_L^e = (\bar{\nu}_e\nu_e)_L + (\bar{e}e)_L$$

and so on.

With $\sin^2\theta_W = 1/4$, the new Gargamelle data (enhancing $(\nu_\mu e^-)$-cross section by a factor around 7 over the $SU(2)_L \times$ U(1)-prediction) seems to need $\alpha^2 \approx 1/2$, giving for the ratio of the masses and couplings:

$$(g_1/m_Y) \approx (g_0/m_Z)$$

For $[\sigma(\nu_e e \to \nu_e e)/\sigma(\bar{\nu}_e e \to \bar{\nu}_e e)]$, the prediction is:

$$= \frac{16\,(\sin^2\theta_W + \alpha^2)^2 + 12\,(\sin^2\theta_W + \alpha^2) + 3}{16\,(\sin^2\theta_W + \alpha^2)^2 + 4\,(\sin^2\theta_W + \alpha^2) + 1}$$

Thus compared to $SU(2)_L \times U(1)$-prediction there is an expected enhancement of $\sigma(\bar{\nu}_e e)$ by a factor of 13/3 while for $\sigma(\bar{\nu}_\mu e)$ there is an expected enhancement by a factor of 3.

The most direct theoretical account of the new neutral current would be in terms of a new local U(1) symmetry. This symmetry could act on a single complex scalar field and the simplest form of Higgs mechanism would break the symmetry and generate a mass for the corresponding vector. Hence a new massive neutral Higgs scalar would appear.

However, as already discussed the form of the new current most likely signals the appearance of a non-Abelian leptonic colour symmetry. For example, if this group is SU(2) then the colour-diagonal current $N^e - N^\mu$ would belong to a triplet; the colour-changing currents being $(\bar{e}\mu)_{L+R} + (\bar{\nu}_e\nu_\mu)_L$ and its conjugate. Such a scheme can be implemented, for example, with a pair of Higgs triplets $(1,3)_0$—in addition to the standard model Higgs doublet $(2,1)_1$. One can set up a potential which causes the Higgs triplets to develop expectation values, u and v with $u^2 \gg v^2$ and $\mathbf{u}.\mathbf{v} = 0$. The gauge triplet $\mathbf{Y}_1, \mathbf{Y}_2, \mathbf{Y}_3$ thereby acquire masses proportional to u^2, $u^2 + v^2$, v^2 respectively, where $\mathbf{Y}_3$ ($\equiv Y$ of the previous model) couples to the colour diagonal current. From above, $m_Y^2 \sim g_1^2 \cdot v^2 \sim (g_1^2/g_0^2) m_Z^2 \sim$ (50–100) GeV^2. $\mathbf{Y}_1$, $\mathbf{Y}_2$, couple to the real and imaginary parts of the colour-changing currents. It is necessary that $u \gg v$ to suppress the colour changing effects like $\mu^+e^- \to \mu^-e^+$ and $\mu \to$ eee. From the present limits on the suppression of these processes, one may infer that $(v/u) \lesssim 0.1$.

This model contains three new Higgs scalars, all neutral and with masses of two of these proportional to u^2 and the third proportional to v^2. These particles do not couple to leptons and will therefore be difficult to create and detect. It is important that the new Higgs fields proposed here are flavour neutral. There will be no mixing with the standard SU(2)×U(1) system and thus no semileptonic effects. So far the treatment assumes the left-handed flavour-symmetry $SU(2)_L \times U(1)$. The main results here based on the idea of nonabelian leptonic colour are, of course, unaltered if we extend the local symmetry to the anomaly free left-right symmetric[3] flavour and colour-gauge structure

$$[(SU(2)_L \times SU(2)_R \times U(1)_{L+R}]_{\text{flavour}}$$
$$\times SU(3)_{L+R}^{\text{quark colour}} \times SU(2)_{L+R}^{\text{lepton-colour}}$$

with the simultaneous introduction of right handed neutrinos[3]. In this case left as well as right handed leptons, that is $(\nu_e, \nu_\mu)_{L,R}$ and $(e^-, \mu^-)_{L,R}$, would form doublets of $SU(2)^{\text{lepton-colour}}$ leading to an unambiguous vectorial current $[(\bar{\nu}_e\nu_e + \bar{e}e) - (\bar{\nu}_\mu\nu_\mu + \bar{\mu}\mu)]_{L+R}$ coupled to $\mathbf{Y}_3$. This will be discussed in detail elsewhere.

If there are three leptonic colours (e, $\mu\zeta$) to rhyme with three quark-colours, the gauge group may be as large as

$$G_{\text{flavour}} \times SU(3)|_{\text{quark colour}} \times SU(3)|_{\text{leptonic colour}}$$

$SU(3)|_{\text{leptonic colour}}$ would contain an additional neutral gauge meson coupling to $(N^e + N^\mu - 2N^\zeta)$. This might also be relatively light (that is of comparable mass to Z^0 and $\mathbf{Y}_3 \approx$ 100 GeV). If this is the case, some of the rate predictions for the purely leptonic model would be altered.

With five (or even possibly six) quark flavours known at present, symmetry structures of the form suggested above may naturally call for left–right colour–flavour symmetric unifying symmetries[4] of the form [SU(6)][4]. (A minimal unifying symmetry [SU(5)][4] suffices to incorporate the idea of nonabelian

leptonic colour with e and μ having different colours. Both $[SU(6)]^4$ and $[SU(5)]^4$ need the presence of mirror fermions for the cancellation of anomalies[4].)

The important point is that within a unifying symmetry structure containing $G_{flavour} \times SU(6)_{(quark+lepton)-colour}$, the leptons must have charge sequence given by $(\nu_e, \nu_\mu, \zeta^+)$ and (e^-, μ^-, ζ^0) corresponding to leptons of up and down flavours. In other words, the three leptons of a given flavour cannot all have the same charge (unlike the case of fractionally charged quarks). This is because $Q = Q_{flavour} + Q_{lepton\,colour} + Q_{quark\text{-}colour}$. If quarks are fractionally charged $Q_{quark\text{-}colour} = 0$; as leptons having the same flavour quantum-numbers as quarks have integer-charges, $Q_{lepton\text{-}colour}$ must contribute to lepton-charges. Since Q is made up of traceless generators, not all three leptons of a given flavour can have the same charge.). This in turn, implies that (ν_τ, τ^-) must be given either electronic or muonic colour (rather than ζ-colour) if τ^- is shown to have $V-A$ interaction. Note that within a nonabelian structure of the form $SU(6)_{flavour} \times SU(6)_{colour}$, there is the additional purely leptonic neutral current $(N^e + N^\mu - 2N_\zeta)$, plus a semi-leptonic current $(N^e + N^\mu + N^\zeta - N_q)$ where N_q involves sum over all three colours.

Professor S. Weinberg has informed us that he has worked out a similar model to our purely leptonic one. J.C.P. has been supported by the NSF under Grant No. GP 4366 2X and S.R. has been supported by the Royal Society of London.

J. C. PATI

Department of Physics,
University of Maryland,
College Park, Maryland

S. RAJPOOT
ABDUS SALAM

International Centre for Theoretical Physics, Trieste, Italy
and
Imperial College, London SW7, UK

J. STRATHDEE

International Centre for Theoretical Physics,
Trieste, Italy

Received 25 April; accepted 8 May 1978.

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Anionic copolymerisation of elemental sulphur

FEW elements are able to form long polymeric chains. This is a feature of carbon atoms, and carbon polymers are usually stable because of the high energy required to break the carbon–carbon single bond and because the further depropagation of the macroradicals, formed by this homolytic splitting of the chain, would usually require additional energy. Here we describe a method for preparing copolymers of sulphur with propylene sulphide; this new method, based on the direct anionic copolymerisation of elemental sulphur is using an ability of sulphur atoms to give polymeric chains.

Elemental sulphur, the eight-membered unstrained ring, has long been known[1] to be able to polymerise on heating. Nevertheless, the polymeric polysulphur:

$$p\,\boxed{S_8} \rightleftharpoons \cdots\!-\!S_n\!-\!\cdots \qquad (1)$$

where $n = 8p$ is thermodynamically unstable at room temperature (equilibrium (1) shifted to the left-hand side) and easily converts back to the elemental octasulphur. This is because the sulphur–sulphur bond in polysulphides has a much lower bond energy than carbon–carbon bonds (~ 30 kcal mol^{-1} compared with 80 kcal mol^{-1} in aliphatic chains, like polyethylene). More importantly, the macroradicals formed on the splitting of the chain:

$$\cdots\!-\!S_x\!-\!S^{\cdot} \qquad (2)$$

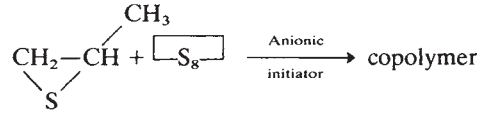
can depropagate at room temperature by an unzipping mechanism, to give the elemental monomeric octasulphur:

$$\cdots\!-\!S_xS^{\cdot} \rightarrow p\,\boxed{S_8} \qquad (3)$$

where $x + 1 = 8p$.

Interruption of the linear chain of sulphur atoms by introducing, for example, carbon atoms should stop this undesirable depropagation. Many previous attempts to obtain high-molecular weight copolymers of elemental sulphur have been unsuccessful, because of the large difference of the reactivities of the carbon- and sulphur-free radicals, and only low-molecular weight oligomers could be prepared in this way[2].

We have recently, for the first time, successfully applied an anionic process to the preparation of the high molecular weight ($\bar{M}_n$ up to 5×10^4) copolymers of elemental sulphur with a number of monomers[3]; in this paper we describe the first of these systems, namely the anionic copolymerisation of 1,2-propylene sulphide (I) with S_8 (II):



(4)

The sulphur content in these coplymers approaches 90%. Copolymers, even with such a high sulphur content are soluble and elastometric; films cast from polymer solutions retain transparency after storage for 4 yr. This indicates that the unzipping depropagation, which could have led to the formation of crystalline II, has been eliminated in copolymers by stopping the depropagation at the first comonomer unit.

As will be shown below, formation of true copolymers with statistical distribution of the polysulphide units has been established by ^{1}H and ^{13}C-NMR studies.

To our knowledge, this is the first report of the successful copolymerisation of S_8 to give stable high-molecular weight polysulphides. Polymers of related structures have long been known, however, prepared by polycondensation of alkali metal polysulphides and α,ω-dihaloalkanes[4].

Copolymerisation was carried out in homogeneous solutions of I and II in benzene or toluene solvents above 60°C under N_2 atmosphere. In typical conditions concentrations of I and II were between 0.5 and 1.5 M, that is, the solutions contained

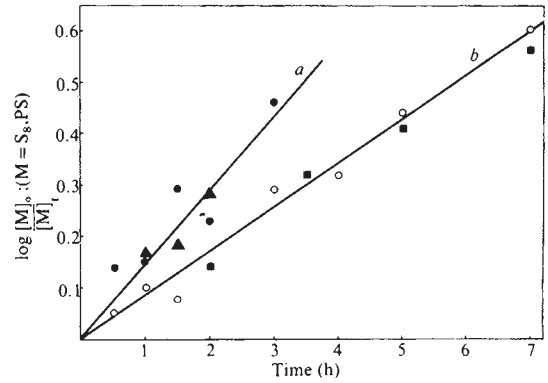


Fig. 1 Kinetics of copolymerisation of propylene sulphide: *a*, with elemental sulphur; *b*, in toluene solvent at 90°. ■, Propylene sulphide at 0.8M with S_8 at 0.4M (▲). ○, Propylene sulphide 0.8M with S_8 at 0.2M (●). 2 mol of $CdCO_3$ was used as initiator in both runs.

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10–30% of monomers in the starting solutions. At higher starting concentrations of II, solutions were initially optically turbid, until a certain conversion was reached. Conversion of I was determined by gas–liquid chromatography and conversion of II gravimetrically. Kinetic curves showing the course of copolymerisation are presented in Fig. 1.

According to Fig. 1, conversion of both comonomers proceeds with comparable rates and, therefore, there is no significant enrichment of the system with either of the monomers during the copolymerisation.

Microstructure of copolymers was studied by ^{1}H and ^{13}C-NMR methods. Figure 2 shows a comparison of the ^{13}C spectra of a homopolymer of propylene sulphide with that of copolymers, having an averaged composition $-CH_2-CH(CH_3)-S_x$ (where $\bar{x} = 1.3$, 2.0, and 5.0).

The ^{13}C-NMR spectrum of a homopolymer, according to previously published data[5], consists of four singlets: CH_3, δ 20.7; CH_2, δ 38.3 and CH, δ 40.4 and 40.1. The two signals from the $\geqslant$CH groups are due to the meso and racemic placements in the corresponding dyads[6].

In the ^{13}C-NMR spectrum of a copolymer the singlets due to the homopolymer are preserved although the fine structure of CH is lost, but their intensity becomes much lower than the intensity of the new singlets appearing in the regions of all of the three carbon atoms. Thus δ 19.4 for CH_3, δ 39.1 for CH_2, and δ 45.6 and δ 45.8 for CH appear in addition to the singlets of a homopolymer units. The new singlets in copolymers are therefore due to the sequences in which the propylene units are divided by the polysulphur bridges of various length:

$$-CH_2-CH(CH_3)-S_x-$$

where $x = 2, 3 \ldots$.

Indeed, it is known that the ^{13}C-NMR chemical shifts for the dialkylpolysulphides can be distinguished separately for up to nine sulphur atoms in the polysulphide chain[6] at high-resolution conditions. The longer the polysulphide bridge, the more downfield shifted the corresponding signals become. It is remarkable that there are at least three signals for a copolymer with $\bar{x} = 5$ in the areas of CH_2 and CH absorption. This means that copolymers do not merely contain S_1 and S_9 units, which would result from a simple addition of both comonomers, but that there is a certain distribution of length of the polysulphide units. It is possible that at higher resolution chemical shifts for C atoms embraced by polysulphide chains of various length will be determined specifically.

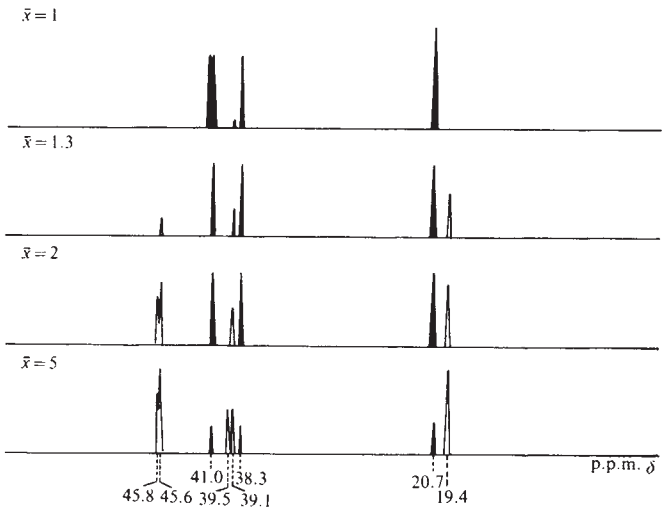

Fig. 2 ^{13}C ^{1}H-NMR spectra of polypropylene sulphide and of copolymers of propylene sulphide with sulphur. The average number of sulphur atoms $\bar{x}$ per one propylene unit is indicated. Black triangles correspond to absorptions due to propylene units separated by one S atom.

Formation of polysulphide units differing in length can be explained by a scheme of copolymerisation involving, in addition to the usual set of homo- and cross-propagations, a scrambling reaction, in which thiolate anions attack the already formed linear polysulphide units in the copolymer chains:

$$\cdots-CH_2-CHCH_3-S_p^- + \begin{array}{l} S-S_x-\cdots \\ | \\ S-S_y-\cdots \end{array} \xrightarrow{k_s} \begin{array}{l} \cdots-CH_2-CH(CH_3)-S_{x+p}-\cdots \\ + \\ \cdots-S_{y+2}^- \end{array} \quad (5)$$

(cation is omitted).

Studies of the kinetics of the CN^- anion reaction with cyclic octasulphur S_8 and linear polysulphides show that the bimolecular rate constant of the latter reaction is much higher than that of the former process[7]. Thus, the copolymer composition, at least at the later stages of copolymerisation, can be governed by the scrambling process, proceeding with a rate constant k_s, which can be much higher than the constants of the individual homo- and cross-propagations.

Thus the anionic copolymerisation of elemental sulphur produces true copolymers with a high S content and fairly high molecular weight (such as 5×10^4). The copolymers with propylene sulphide, described here, are stable at atmospheric conditions and do not depolymerise even after several years. This unusual copolymerisation may provide the basis of a new area of high-polymer synthesis involving elemental sulphur.

STANISŁAW PENCZEK
ROMUALD ŚLĄZAK
ANDRZEJ DUDA

Polish Academy of Sciences,
Centre of Molecular and Macromolecular Studies,
90–362 Łódź, Boczna 5, Poland

Received 19 January; accepted 23 March 1978.

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Halokinesis and thermal convection

THEORETICAL, numerical and experimental studies[1–3] have successfully modelled realistic analogues of natural salt domes which have risen from deep source layers through formerly superincumbent layers of rock, by a process known as halokinesis. In such studies the deformation has been approximated to the gravitational stabilisation of Rayleigh-Taylor instabilities in which dense viscous cover layers originally overlay a less dense effectively viscous salt layer. Various examples of salt deformation have been reported in the past decade which seem to have involved salt too shallow for the overlying sediments to have either compacted to densities greater than that of crystalline salt[4] or to load the (actually plastic) salt above its assumed yield point[5]. Thus in part of the North Sea the Zechstein salt seems to have started doming in Muschelkalk times when buried by only 610–760 m of (possibly soft) argillaceous sediments[6]. Similarly, in part of the Mississippi basin, salt seems to have moved beneath an overburden thickness of 300–600 m (ref. 4). Furthermore, small salt domes seem to be actively

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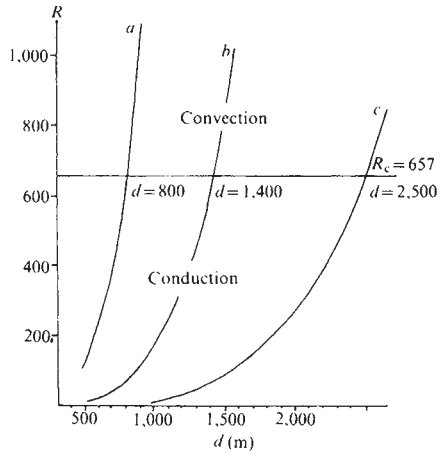


Fig. 1 Rayleigh number (R) plotted against thicknesses (d) of crystalline halite layers with various viscosities in a vertical thermal gradient of 30 °C km^{-1}; g, 9.81 m s^{-2}; α, 10^{-3} deg^{-1}; β, 30×10^{-3} °C m^{-1}; κ, 1.7×10^{-5} cal m^{-1} s °C. a, $\mu = 10^{12}$ m^2 s^{-1}; b, $\mu = 10^{13}$ m^2 s^{-1}; c, $\mu = 10^{14}$ m^2 s^{-1}.

developing beneath only 150–300 m of valley-fill alluvium in the 2,000–2,600 m thick tabular Luke salt body in Arizona[7,8]. Such cases of halokinesis, being premature in terms of the likelihood of unstable compositional density gradients, suggest that one or more factors previously neglected may be involved in at least some salt deformations[4]. Here the degree of mechanical instability due to geothermal gradients within thick layers of salt and in rock sequences with salt at the bottom is examined. I find that heat may have a more significant effect on halokinesis than that of merely reducing the strength of salt[5], and that cyclic thermal convection may occur within some salt bodies and that some salt domes may develop as thermal plumes.

Thermal convection affects simple fluid layers when their Rayleigh number (R) exceeds a critical value depending on the boundary conditions[10]. For horizontal layers of homogeneous incompressible Newtonian fluids $R = g\alpha\beta d^4/\kappa\mu$ where g is the acceleration due to gravity, β the uniform vertical temperature gradient, d the thickness of the layer and α, κ and μ are, respectively, the coefficients of volumetric expansion, thermal diffusivity and kinematic viscosity.

Figure 1 shows the variation of Rayleigh numbers for layers of pure crystalline salt with various thicknesses and effective viscosities in a temperature gradient of 30°C km^{-1} (current North Sea values of 44–18°C km^{-1} are given in ref. 11) and with the other parameters as listed. The critical Rayleigh number shown on Fig. 1 ($R_c = 656$) is where the top and bottom boundaries are free but inflexible[10].

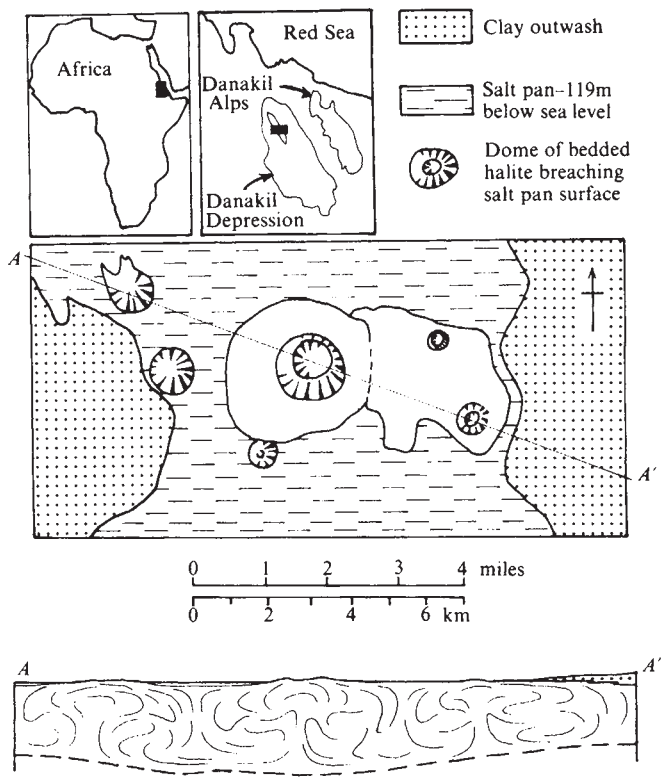
The volumetric expansion of halite due to heat is greater than its compressibility due to pressure. In a thermal gradient of 30°C km^{-1} and at a depth of about 5,000 m, for instance, halite would expand 2% because of the 170°C temperature[12] and shrink 0.5% because of the 1 kbar pressure[5]. Crystalline salt is obviously not an incompressible Newtonian fluid but is commonly taken to have an effective viscosity of about 10^{17} stokes = 10^{13} m^2 s^{-1} (ref. 12). Figure 1 is based on a gross simplification of the actual situation but implies that salt bodies may undergo sustained internal cyclic convection in areas of steep but not unusual geothermal gradient if they exceed thicknesses of about 1,500 m. This is independent of any superincumbent load and convection might occur in very shallow salt bodies if the normal yield strength of the salt were to be drastically reduced by additions of fresh water[13].

Figure 2 shows a sketch map of part of a modern salt pan in a region of high heat flow near Dallol in the Danakil Depression of Ethiopia[14]. Five or six domes of bedded halite breach the accumulating surface crust of salt and three of these are shown in my interpretative cross section on Fig. 2. Seismic investigations indicate that the salt is at least 2,200 m thick and has a complicated internal structure with reflecting interfaces dipping in opposite directions at different levels[15]. Hot brine springs (100°C and 130°C) which rise in two of these domes fluctuate seasonably and might represent recirculated groundwater supplied by occasional freshwater floods[14] which could render the salt sequence particularly mobile. Such domes of bedded salt apparently rising through more salt are unlikely to be gravity structures due to compositional density instabilities but might be examples of gravity structures arising because of thermally induced instabilities.

If a soft sedimentary sequence accumulates on top of a layer of salt, only those parts of the cover deeper than about 600–1,000 m are likely to have compacted to densities greater than that of salt[4]. As most other rocks conduct heat about 10 times more slowly than does crystalline salt, the cover is likely to act as thermal insulation above a flexible interface before it becomes an unstable load. The introduction of a flexible boundary would enhance any instability (and reduce the relevant critical Rayleigh number) because the unstable fluid is able to adopt its optimum motion by penetration into bounding layers which could otherwise be stable[16,17]. A convecting salt layer may therefore send columns of relatively hot salt upwards into a soft and slightly less dense cover above a flexible interface. The cases of salt domes rising into very thin covers may be examples of such deformations and it should be possible to test such a model in the Danakil Depression and in Arizona where the domes still seem to be developing[7,8].

Even in areas of classical halokinesis the geothermal gradients are likely to have added an extra component of gravitational instability to situations in which more dense layers originally overlay salt layers. A crude attempt to assess this factor for a 'characteristic' salt dome situation is given by neglecting the undoubtedly large range of material properties involved in such sequences. Thus, single representative values

Fig. 2 Domes of bedded halite emerging through a crust of reprecipitated salt on the pan in the Danakil Depression, Ethiopia (from an aerial photograph in ref. 14). The cross section shows a visualisation of possible thermal convection cells.



have been chosen for each property of the complete characteristic system. If

$$\text{Thickness of cover} = 3{,}000\ \text{m}$$

and

$$\text{Thickness of salt} = 1{,}000\ \text{m}$$

hence

$$d = 4{,}000\ \text{m}$$
$$g = 10\ \text{m s}^{-2}$$
$$\alpha = 10^{-3}\ ^\circ\text{C}$$
$$\beta = 30^\circ\text{C km}^{-1} = 3\times10^{-2}\ ^\circ\text{C m}^{-1}$$
$$\mu = 10^{18}\ \text{Stokes} = 10^{14}\ \text{m}^2\ \text{s}^{-1}$$
$$\kappa = 10^{-6}\ \text{cal m}^2\ \text{s}^{-1}$$
$$R = g\alpha\beta d4/\kappa\mu$$
$$= 768$$

Little is known of the relevant material properties of rocks and the approach used is so crude that the Rayleigh number derived may be in error by several orders of magnitude. Nonetheless, it is sufficiently high to suggest that the unstable density gradient induced by the natural heat flow adds significantly to the compositional density inversion previously considered solely responsible for the development of most salt domes.

Regions in which the geothermal gradients are low when salt layers are buried therefore require thicker cover before halokinesis can start compared to regions in which the geothermal gradients are high. Until more is known of the parameters of such systems it is not worth taking into account possible complications such as variable sedimentation rates or heat changes within the sequence due to friction, chemical reactions[18], radioactivity or groundwater circulation in porous covers[19].

The rise of salt domes or walls to shallow levels up the centres of polygonal or rectilinear movement cells involving the whole rock sequence, changes both the mechanical stability and vertical thermal diffusivity of the complete system. A sufficient volume of salt will rise to stabilise any compositional density instabilities and the regional heat will leak up the highly conductive salt columns or walls. Because the simple rise of salt structures through a cover can mechanically stabilise rock piles in respect to both compositional or thermally induced unstable density gradients there is no tendency for cyclic motion to occur in mixed rock sequences as there would be for convective motions inside salt bodies with inflexible boundaries.

Proposals for the storage of high grade radioactive waste in bodies of salt[20] should consider that the salt may transfer the enormous amounts of heat generated by such waste by convection, in addition to radiation, and conduction.

I thank E. A. Rogers for stimulating discussions.

C. J. TALBOT

Department of Geology,
University of Dundee,
Scotland, UK

Received 2 February; accepted 19 April 1978.

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Multiple spinel–garnet peridotite transitions in upper mantle: evidence from a harzburgite xenolith

THE transition between spinel- and garnet-peridotites, caused by the reaction olivine + garnet ⇌ orthopyroxene + spinel, has been investigated by petrographic study of xenoliths[1,2] and by reconnaisance experiments on phase equilibria. We report here an unusual spinel-garnet harzburgite xenolith from the Liqhobong kimberlite (north-west pipe), Lesotho, that shows evidence of a complex history.

The mineral proportions of six thin-sections of the xenolith (labelled BD2093) vary as follows: olivine 50–70%, mean 60%; orthopyroxene 45–25%, mean 34%; garnet 1–7%, mean 5%; spinel ~1% for all. Most olivine and orthopyroxene grains are less than 2 mm across whereas the garnet grains, whose shape varies from irregular to euhedral (Fig. 1*a*), may be up to 3 mm in diameter. All silicate grains are strongly fractured with no obvious bending, and alteration along grain boundaries obscures some textural relationships. The spinel grains, red in colour and usually less than 0.5 mm, are very irregular (Fig. 1*a*, *d*, *f*) and scattered erratically without any preferred association with orthopyroxene. Spinel also occurs intergrown with orthopyroxene as irregular bands in one garnet grain (Fig. 1*a*). Spinel–pyroxene symplectites do not occur around the garnet, and there are no holly-leaf clusters as in the xenoliths from the Green Knobs diatreme[2].

The garnets are concentrically zoned (Fig. 1*a*, *b*; Table 1) from a mauve Cr-rich core to a wide uncoloured Al-rich rim, and there is a narrow Al-rich band around the spinel–orthopyroxene inclusions. The composition profile (Fig. 1*c*) for the section AB in Fig. 1*a* is interpreted as the result of diffusional transfer of Cr from garnet to secondary spinel coupled with appropriate migration of other elements. The Cr content of orthopyroxene increases as spinel crystals are approached (Fig. 1*d*, *e*). A euhedral orthopyroxene grain occurs inside the unzoned core of a garnet crystal, and is rich in both Cr and Al (Table 1). The Al_2O_3 content of orthopyroxene grains ranges from 1.5 to 2.4 wt %, but does not correlate with texture or mineral association. The spinel inclusions in the garnet have higher Cr/Al ratios than those remote from garnet (Table 1), and spinels growing at the edge of garnets (Fig. 1*a*) have intermediate values; this apparently indicates decreasing efficiency of diffusion with increasing distance of transfer from the garnet to the secondary spinel.

The silicate minerals are exceptionally rich in Mg and low in Ca even when compared with similar phases in kimberlitic xenoliths and diamond inclusions. The garnet may be classified as a low-calcium chrome-pyrope[3,4], but the Cr_2O_3 range (1–6 wt %) only partly overlaps the range of 3–15 (mean 8) established by Dawson and Stephens[3] for 77 garnets from kimberlitic environments. Only two of these 77 garnets were from xenoliths, and in both rocks, the other silicates were serpentinised, thereby ruling out further compositional information. Many of the 77 garnets occur as inclusions in

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diamond, but both the present olivine ($Fo_{95.6}$) and orthopyroxene ($En_{95.7}$) are more magnesian than the ranges ($Fo_{91.5-93.4}$, $En_{90.5-94.5}$) listed by Sobolev[5] for a diamond host. Furthermore, the orthopyroxene is much lower in CaO (0.05 wt %) than the range (0.24–0.45) for diamond inclusions.[5] The spinel lies close to the compositional range for harzburgitic specimens[6] but is displaced toward higher Mg/Fe while its Cr/Al ratio is much lower than that for chromite inclusions in diamond.

A granular spinel harzburgite (PHN2600), also from Liqhobong (S. blow)[7], demonstrates a similar Mg-rich, Ca-poor environment (olivine, $Fo_{95.5}$; orthopyroxene, $En_{95.9}$. CaO 0.08) but its spinel is richer in chromium (Cr_2O_3 40.1, Al_2O_3 30.4); garnet is absent.

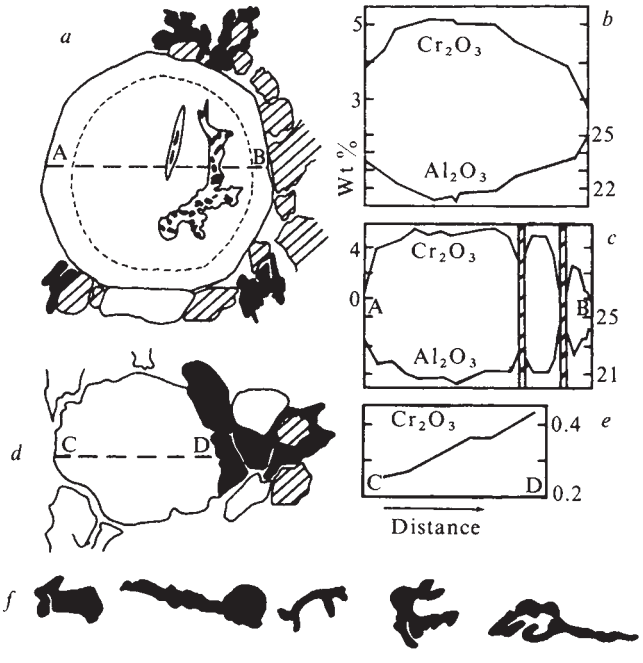
Experimental data on the transition from spinel- to garnet-peridotite generally agree[8–12], but the effects of substitution of Fe^{2+} for Mg, and of Cr^{3+} and Fe^{3+} for Al, are rather uncertain. Furthermore, there is uncertainty on the positions of the isopleths of Al_2O_3 in enstatite (see refs. 13, 14). MacGregor[8,9] explored substitution of CaO, Cr_2O_3, Fe_2O_3 and Al_2O_3 on the reaction olivine + garnet = orthopyroxene + spinel, and Wood[10] experimented on Cr substitution and provided a model calculation for substitution of Fe^{2+} and Ca. Because of uncertainties in experimental data and thermochemical models and absence of clinopyroxene in BD2093, we shall merely refer the Liqhobong assemblage to the line given by MacGregor[9] in his Fig. 5 for a spinel with Al : Cr : Fe ~ 62 : 32 : 6. A similar conclusion results from combining the data of Wood[10] and MacGregor[11]. Although it is impossible to give an accurate estimate of the temperature and pressure of reaction in the present harzburgite, the low Al_2O_3 content of the orthopyroxene (~2 wt %) suggests a temperature below 1,000 °C (perhaps near 700 °C) while the coexistence of Cr-bearing spinel and garnet may require a pressure in the range 15–25kbar. These suggested conditions would not be inconsistent with metamorphism on a shield geotherm at approximately 70 ± 20 km.

Table 1 Representative analyses for specimen (BD 2093)

	1	2	3	4	5	6	7	8	9
SiO_2	47.0	44.4	42.5	41.7	58.4	57.9	58.7	0.23	0.12
TiO_2	0.01	0.0	0.01	0.01	0.0	0.01	0.0	0.01	0.0
Al_2O_3	2.0	19.7	24.8	0.01	2.42	1.56	1.80	33.6	41.8
Cr_2O_3	0.6	5.84	0.89	0.01	1.15	0.50	0.27	35.9	28.1
FeO	4.0	5.47	4.93	4.59	2.99	2.80	2.91	8.41	7.73
MnO	0.08	0.33	0.28	0.06	0.10	0.11	0.04	0.09	0.09
MgO	45.8	24.7	25.1	53.3	36.4	36.4	37.5	18.9	19.5
NiO	0.26	0.0	0.0	0.38	0.07	0.09	0.16	0.08	0.10
CaO	0.04	0.42	1.12	0.0	0.04	0.02	0.05	0.0	0.0
Na_2O	0.0	0.0	0.0	0.0	0.04	0.03	0.0	0.0	0.0
Sum	99.79	100.86	99.64	100.1	101.62	99.46	101.45	97.22	97.44

1, Estimated bulk composition of BD2093 based on average mineral composition and modal analysis; 2,3, garnet; core and rim of 3-mm crystal; 4, olivine; unzoned grains; 5, orthopyroxene; euhedral grain enclosed by garnet; 6, orthopyroxene; enclosed in garnet and intimately associated with spinel (Fig. 1*a*); 7, orthopyroxene; edge of grain, away from spinel (Fig. 1*d*, point C); 8, spinel; inclusion in garnet, intimately associated with opx (Fig. 1*a*); 9, spinel; in matrix of olivine and opx, remote from garnet (Fig. 1*f*). Electron microprobe analyses with Chicago 1977 wavelength–dispersive procedure; detection of 0.02 (2σ), accuracy 1–5% of amount present for major elements.

Fig. 1 Textures and composition profiles. *a*, Euhedral garnet (2 mm across) with zoned profile AB shown in (*c*). The dashed line shows the strongly zoned rim. Two areas of spinel (black) and orthopyroxene (unshaded) are shown. Surrounding the garnet are spinel, olivine (hatched), orthopyroxene and serpentine filling of fractures. *b*, Typical profile for zoned garnet free of spinel and orthopyroxene inclusions. *d*, Orthopyroxene (1 mm across) with composition profile CD shown in (*e*). X-ray fluorescence was ignored in calculating Cr_2O_3 content. *f*, Shapes of spinel crystals in olivine–orthopyroxene matrix distant from garnet, 0.1–0.2 mm long.



A complex history for the xenolith can be deduced. First, the compositional zoning and textural features of BD2093 harzburgite can be best explained by incomplete transformation from garnet- to spinel-harzburgite, with compositional profiles governed by diffusion. These diffusion profiles in the garnet and orthopyroxene suggest a significant period when the rocks were held at high temperature (not necessarily constant), which has analogues in the common occurrence of diffusion profiles in garnet-bearing rocks from regionally metamorphosed terrains. This contrasts with sharp chemical discontinuities in traverses along pyroxenes developed (in continuity with pre-existing orthopyroxene) in symplectite rims around peridotitic garnets developed during rapid transportation out of their stability field[1]. The inference is that some early attempt at equilibration was achieved in BD2093, although it was possibly terminated by entrainment and transportation of the xenolith in the host kimberlite.

Second, an earlier stage can be inferred from the estimated bulk composition of the xenolith (Table 1, Column 1). Compared with the composition of other peridotitic and eclogitic xenoliths from the upper mantle, together with those of basaltic extrusives, the very low CaO content (0.04 wt %) and the high Mg/Fe ratio (~20) imply an unusual origin for the Liqhobong harzburgites. An original assemblage dominated by olivine (~60%) and low-Ca pyroxene (~35–40%) is indicated. As olivines and orthopyroxenes from mantle-derived peridotite xenoliths typically contain 0.0*x* and 0.*x* wt % CaO, there is no toleration for any significant amount of a Ca-rich mineral such as diopside, and little scope for even a pyrope-rich garnet with low Ca. Perhaps a small amount of Cr-bearing spinel (0–1%) might be present, but the orthopyroxene might carry all the Cr_2O_3(~1.5 wt %) and Al_2O_3(~5 wt %) estimated from the bulk composition and mode.

The proposed olivine–orthopyroxene assemblage might have developed either by cumulation from a low-Ca 'basaltic' liquid or by extraction of basaltic liquid during partial melting of a peridotite. Estimation of the temperature is difficult. Experimental data for the dry Mg_2SiO_4-SiO_2 system[15] show that forsterite and orthopyroxene lie on the solidus at 1,700 °C for 15 kbar and 1,790 °C for 25 kbar, while experimental data for the dry Mg_2SiO_4–$CaMgSi_2O_6$–SiO_2 system[16] show that forsterite and orthopyroxene lie on the solidus at temperatures above 1,640 °C for 20 kbar, whereas diopside-bearing assemblages occur at lower temperatures. The effect of Fe substitution for Mg is unknown, but crude analogy with the phase equilibria for olivine and for pyroxene compositions suggests

that 5% substitution of Fe for Mg would not lower the above solidus temperatures by more than 100°C. Presence of excess water for the Mg_2SiO_4–$CaMgSi_2O_6$–SiO_2 system lowers the temperature of 1,640° C to 1,220 °C (ref. 16), but such an extreme condition is unlikely in view of the low water content of basaltic magmas. Although a precise temperature cannot be established because of lack of knowledge of the composition of the supposed magma, and although there is no obvious control over the pressure, we suggest that the original olivine–pyroxene assemblage formed at a temperature not lower than 1,400 °C and perhaps as high as 1,700 °C unless volatile-rich conditions were involved.

Third, an intermediate stage of solid-state recrystallisation is required to give an olivine–orthopyroxene–garnet assemblage before the final stage of incomplete transformation to spinel harzburgite. Specimen PHN 2600 may have followed the same three-stage sequence with complete elimination of garnet, or alternatively might not have entered the field of garnet peridotite because of its high bulk Cr content, implying a higher transformation pressure, or lower temperature, or both[9]. The higher content of CaO in the 2600 orthopyroxene (0.08 wt %) than in the 2093 orthopyroxene (0.02–0.05 wt %) could result from absence of coexisting garnet in the 2600 assemblage.

Unfortunately, the present data allow no estimate of pressure change, except for the crossing of the garnet–spinel transition. However, the estimates of temperature change are insensitive to uncertainties of ~10 kbar in the pressure, and the temperatures estimated for the original formation of the harzburgite (1,400–1,700 °C?) are considerably greater than those estimated for the solid-state equilibration (700–1,000 °C?) if volatile-rich conditions were not involved in the formation of the primary assemblage. Perhaps the Liqhobong specimens may be a low-Ca analogue of recrystallised calcic and chromiferous cumulates postulated by Clarke and Carswell[17], and may be matched with the subducted cumulates postulated by Ringwood[18] and others. Certainly, the Liqhobong harzburgites add yet more evidence on the complexity of the upper mantle, especially as a result of solid-state disequilibration.

J. B. D. thanks the Royal Society, NERC and University of St Andrews for travel grants. J. V. S. thanks NSF for grant EAR 75-03604 and partial support by the Materials Research Laboratory Program at the University of Chicago. P. J. Wyllie offered advice on the phase equilibria. R. L. Hervig assisted with electron microprobe analyses, and S. H. Smith with drawing.

J. B. DAWSON

Department of Geology, University of St Andrews, Scotland, UK

J. V. SMITH

J. S. DELANEY

Department of the Geophysical Sciences, University of Chicago, Chicago, Illinois 60637

Received 5 January; accepted 22 March 1978.

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Deep shelf and slope terraces off northern Labrador

SUB-BOTTOM profiling with a 3.5 kHz hull-mounted sounding system was conducted along 49 closely spaced north-east–south-west orientated lines over the southern two-thirds of Saglek Bank (Fig. 1) and on the adjacent slope. The echo sounding profiles reported here reveal a striking series of terraces superimposed on what is otherwise a relatively smooth, acoustically reflective surface in water depths ranging from 130 to 400 m (Fig. 2).

To examine trends in terrace distribution (Fig. 3), we mapped the mid-points of all the observed terrace platforms and then projected them onto a vertical plane. Terraces on adjacent profiles were correlated primarily according to depth but also by taking into account characteristic morphology, number of terraces per profile and patterns of vertical and horizontal terrace spacing. Correlated terraces have been numbered sequentially above and below a long, prominent terrace that generally occurs near the shelf break. This long terrace is pronounced on all profiles (except one) between 58°20′ N and 59°25′ N, a distance of over 100 km (Fig. 3).

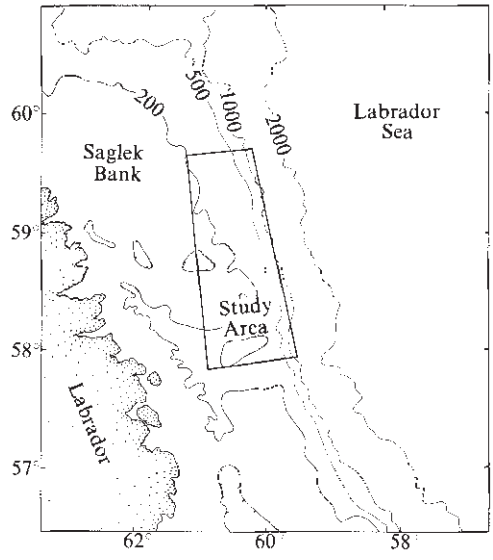

Fig. 1 Location map, northern Labrador Shelf.

Within this zone it averages 260 m deep, ranging from its shallowest occurrence at 235 m near 58°45′ N to 285 m at 59°20′ N and exhibiting a virtually constant northwards tilt of 1:1,000 for over 55 km (Fig. 3). South of about 58°45′ N it gradually assumes a gentle southerly tilt.

The degree of correlation apparent from Fig. 3 suggests a series of long curvilinear features rather than separate slump scars. The occurrence of well defined concentric terraces girdling the small elliptical bank just to the south of Saglek Bank (Figs 1 and 3) also largely eliminates the possibility that the terraces are fault scarps. Seismic profiles (for example, Fig. 4) suggest that underlying strata have not been significantly offset

0028–0836/78/0273—0743$01.00

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or distorted beneath the terraces as would be expected for a slump or fault zone.

The transverse morphology and longitudinal tilt of the Saglek Bank terraces −3 to +7 very closely resemble those of shallowly submerged (<160 m deep) erosion-dominated shorelines such as the 'Nichols,' 'Franklin' and 'Fortune' off the northeastern US[1-3]. They are also in the same range of water depth as, and basically quite similar in form to presumed sea-level terraces observed at 200 m on Cobb Seamount[4] and 250 m off the Bering and South Australian shelves[5].

Terraces −3 to +7 have apparently been cut into a 300-m thick wedge of glacial drift that comprises the bulk of Saglek Bank[6,7] implying that their formation postdates the advent of significant North American glaciation. Their greater departure from the horizontal with increasing depth further suggests that the terraces were formed successively from deepest to shallowest and that the continental margin has simultaneously undergone gradual differential tilting. The tilt of the terraces parallels a northwards dip of subsurface reflectors toward a sedimentary basin off Hudson Strait[8,9], suggesting that tectonically induced subsidence may have contributed to terrace deformation. On the other hand, residual depression created by thick, late Wisconsinan glacial ice grounded in Hudson Straight might explain the tilt.

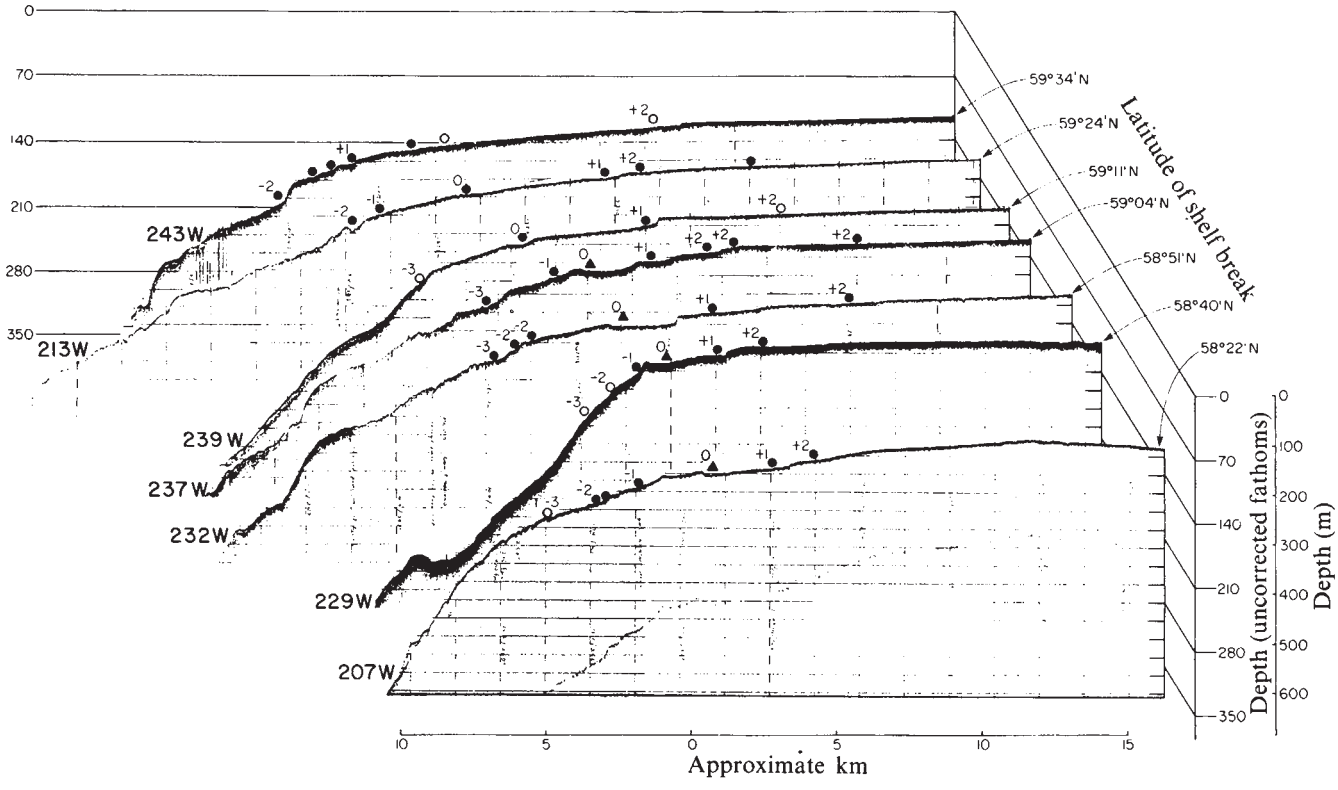
If all the Saglek terraces were produced at or near sea level during the last glaciation, at least 200 m of glacio-isostatic subsidence caused by the collapse of peripheral uplift[10] and 100 m of eustatic sea level lowering are called for. However, less than 80 km away on the Labrador coast, raised deglacial shore line terraces older than 9,000 yr (ref. 11) point to a comparatively small postglacial rebound of about 60 m. These contrasting relative displacements are not at all compatible with Walcott's[10] model of postglacial rebound. A pre-late Wisconsinan age for the terraces must therefore be considered. If speculation about terrace age is constrained by setting reasonable limits on the amount of peripheral bulge[10] and sea-level lowering[12-14], the terraces would indeed seem to be relatively ancient. We shall now examine the implications of an hypothesis that the terraces record pre-Wisconsinan sea levels.

Neogene subsidence of Hamilton Bank on the southern Labrador Shelf has been estimated at ~15 cm kyr^{-1} (ref. 7). Subsidence of Saglek Bank at a similar rate over a period of about 1.7 Myr could account for the present depth of the −3 terrace (~350 m below sea level) if the terrace was formed in conjunction with a low (−100 m) glacial sea level. High, interglacial stands of the sea would not have left recognisable strand lines on a subsiding continental margin because of erosion during subsequent interglacial transgressions.

Cyclical variations in the oxygen isotopic composition of seawater during the past 3.2 Myr are considered to reflect the periodic growth and decay of Northern Hemisphere ice sheets[15]. The data suggest that there has been only minimal deviation from an average periodicity of ~90,000 yr between isotopic glacial maxima (equivalent to sea-level minima) during this period. If the 11 principal terraces on Saglek Bank developed during successive major glacial events, then the period of repeated terrace formation would have lasted just under 1 Myr.

For hypothetical terraces produced during glacial events 90,000 yr apart, a vertical separation of 13.5 m would be indicated for Saglek Bank. The actual vertical separation between terraces ranges from about 10 m to 50 m with terrace spacing from the 0 level to the +7 level averaging slightly less than 20 m (Fig. 3). The observed tendency toward decreasing vertical spacing with time can be explained by either decreasing maximum quantities of glacier ice stored on land or by deceleration of crustal subsidence or some combination of the two effects.

Fig. 2 Representative 3.5 kHz echo sounder profiles of terraces on the shelf and upper slope of Saglek Bank. Terraces correlated for significant distances along the margin, using as a data base a total of 49 profiles, are numbered sequentially above (+) and below (−) a prominent shelf edge terrace (0). Profile numbers at the left are for identification in Fig. 3. The perspective shown is a mirror image of the actual margin. 'Terrace with barrier' refers to small hills at the seawards end of some terrace platforms. ●, Definite terrace; ○, probable terrace; ▲, terrace with barrier.



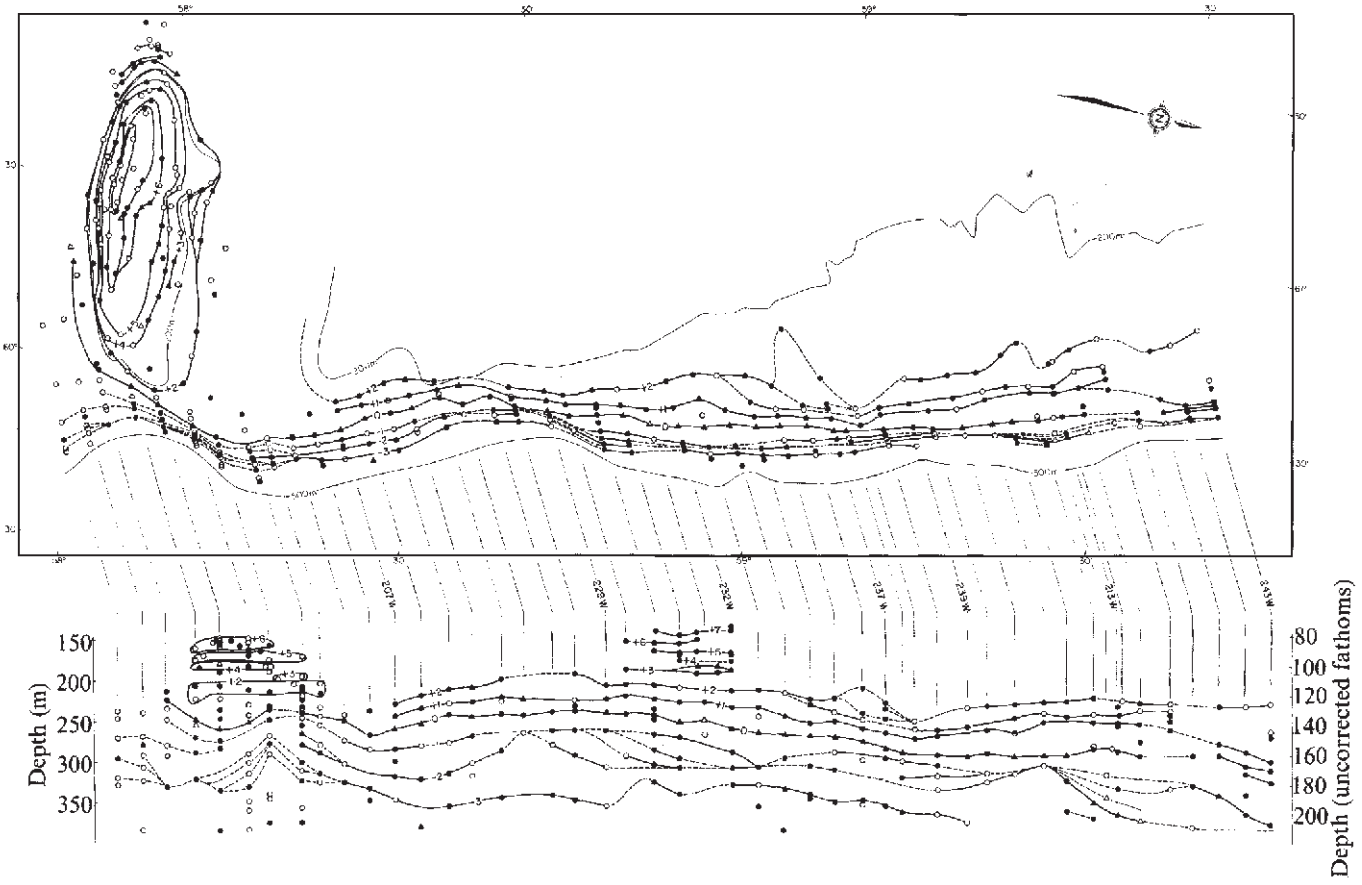


Fig. 3 Correlation of terraces appearing on 49 echo sounder profiles run by MV Martin Karlsen. Terraces depicted in the lower, vertical section have been projected on a vertical plane orthogonal to the ship tracks shown in map view. The 200 m and 500 m isobaths are for perspective only and are approximate. Terrace envelopes +2 to +6 (at left) encompass the scatter of depths along terraces encircling the small bank. Terraces +3 to +7 in the centre of the diagram are not depicted in map view because they are associated with scattered bathymetric highs. Terraces are generally well defined between profiles 270° W and 213° W. Outside this zone, to the north and south, the long prominent shelf edge terrace (0) and most of the other terraces are less well defined and apparently more sharply tilted so that correlations between nick points on adjacent profiles are less certain. It is arguable that individual terraces on Saglek Bank become increasingly difficult to trace as Hudson Strait (north of 60° N) and the transverse shelf channel centred at 58°15′ N are approached. Several possible but as yet untested explanations include underlying strata of variable competency, the presence of palaeo-river mouths, the close proximity of a localised glacial ice lobe at the time of terrace formation and differential masking by later sediment deposition or submarine erosion. ●, Definite terrace; ○, probable terrace; ▲, terrace with barrier; ▼, probable terrace with barrier.

The highest portion of Saglek Bank is now submerged to a depth of about 130m. The +7 terrace occurs at about 137 m and may or may not represent the late Wisconsinan glacial maximum at 18,000 BP. Glacio–eustatic draw-down of only 100 m (ref. 12) would imply that the late Wisconsinan event and two preceding glacial events probably went unrecorded on the bank. The age of terrace +7 could, therefore, be as great as $\sim 3 \times 10^5$ yr (Illinoian). However, if the late Wisconsinan low sealevel stand was 130 m lower than present sea level[13], the +7 terrace may have formed then. The bank margin in the study area was within about 250 km of Laurentide Ice in the lowlands west of the coastal mountains and in Hudson Strait[16] and could thus have been subject to either minor peripheral glacio–isostatic upwarping or downwarping of several metres depending on the precise values of crustal and glaciological parameters[10]. A viewpoint which considers the effect of ice sheet mass on the shape of the geoid predicts practically no glacial sea-level lowering relative to the present for northern Labrador[14]. If this interpretation is accurate, terrace +7 could conceivably have been formed $\sim 9 \times 10^5$ yr BP assuming zero glacio–isostatic deformation and an average 15 cm kyr^{-1} crustal subsidence rate. Terrace −3 would in this case be ~2.0 Myr old and the underlying glacial drift older still. Considering that significant ice-rafted sediment deposition in the Labrador Sea was underway by 3.0 Myr BP (ref. 17) even these maximum estimates of terrace age are not unrealistic.

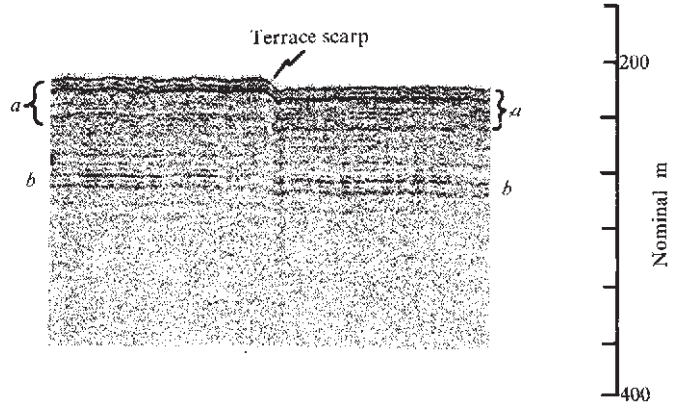


Fig. 4 Air-gun seismic profile over the +2 terrace. *a*, The pulse returns from the sea-bottom; *b*, sub-bottom reflector. The small offset in *b* below the terrace scarp is interpreted as an apparent (velocity) displacement only. The distance spanned by the section is approximately 1 km.

We thank the Canadian Hydrographic Service for the navigational control provided on the cruise on MV Martin Karlsen and also C. Beaumont, A. C. Grant, L. H. King and B. MacLean for discussions.

RICHARD H. FILLON
R. ALLIN FOLINSBEE
RICHARD PALMER

Atlantic Geoscience Centre, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

Received 29 December 1977; accepted 19 April 1978.

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Off-ridge volcanism and seafloor spreading in the Shikoku Basin

THE nature of the crust and origin of marginal basins of the western Pacific continue to be of widespread interest to earth scientists. Recent advances in mapping magnetic anomalies and drilling basement rocks in these areas have suggested that marginal basins are floored by ocean crust[1] and formed by conventional seafloor spreading processes from a mid-basin ridge[2–4]. Our drilling results in the Shikoku Basin during Leg 58 of the Deep Sea Drilling Project reported here indicate that this basin formed as a consequence of back-arc spreading, but that the original simple spreading hypotheses need reappraisal because the crustal stratigraphy and so-called basement age relationships are more complex than expected.

We drilled three sites in the Shikoku Basin (Fig. 1). Site 442 is located about 50 km to the west, and Sites 443 and 444 are about 95 km to the east of a postulated extinct spreading centre[5,6] oriented NNW–SSE. The sedimentary section at all three sites consists of hemipelagic clays (Fig. 2) on the distal part of clastic wedges, which seismic reflection data indicate thicken towards the Kyushu–Palau Ridge to the west and towards the Iwo Jima Ridge to the east[7,8]. We drilled 163 m of basalt at Site 442, 120 m at Site 443, and 44.5 m at Site 444. At all three sites, the sediments were intruded by massive diabase sills and sub-sediment basalt flows. Sediment was found overlying pillow basalts only at Site 442. Pillow lavas were not found at Site 444 and the hole bottomed in diabase intrusives. The intrusives and pillow lavas consist of aphyric, plagioclase phyric, and plagioclase–olivine phyric basalt. Olivine occurs as a groundmass or phenocryst phase in about 50% of the basalts but was absent at Site 442. Except for a somewhat high degree of vesiculation (up to 30%) the basalts and diabase all seem petrographically to be typical mid-ocean ridge basalts.

The identification of the sills and sub-sediment flows at the three sites was based on their coarse grain size, their massive nature, the predominance of fine-grained chill zones rather than glassy chill zones, and the presence of baked sediment at upper and lower contacts. The baked sediment was recognised as such by its composition and darker appearance, and from shipboard analysis of pyrolysed organic carbon in the sediment near the sill contact.

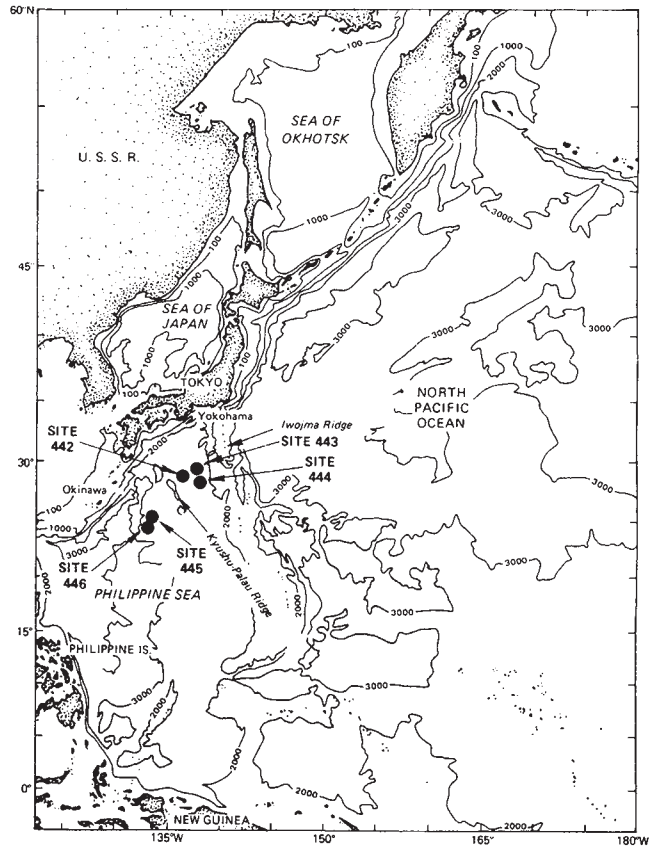

Fig. 1 Map of northern Philippine Sea showing location of sites in Shikoku Basin.

The oldest sediments at Site 442 are red pelagic clays found above pillow basalts but below 59 m of intrusives; they are dated palaeontologically at 18–21 Myr BP which agrees with the postulated magnetic 6 anomaly age[5,6] (Table 1). The age of the sediment immediately above the intrusives is 15–17 Myr BP, which is younger than the postulated magnetic anomaly. At Sites 443 and 444, which are located 100 km apart on the same postulated magnetic anomaly (6A, about 21.5 Myr BP), the oldest sediment occurs above basalt intrusives and is palaeontologically dated at 14–15 Myr BP (Table 1). A 10.5-m thick sill, for which we estimate an age of 13–14 Myr BP was found in younger sediments at Site 444. The minimum ages for the basalts at the bottom of Holes 443 and 444 are far younger than those expected from the magnetic anomaly age based upon a symmetrical spreading model[2,5,6]. It is possible, however, that the sills are considerably younger than the true basement ages at Sites 443 and 444.

The natural remanent magnetism of the basalts is sufficiently stable to have held its original direction and polarity. Its intensity ranges from 1×10^{-3} to 1×10^{-2} G cm^{-3}. At Site 443, its polarity is reversed throughout the hole; at the other two sites, we found a succession of normal and reversed polarities. These results contrast with the positive magnetic anomaly observed at these three sites, and indicate that the uppermost layer of the ocean floor in this area is not responsible for the magnetic anomalies. The basalts we drilled may be younger than the formation of the deeper crust, causing the linear magnetic anomalies.

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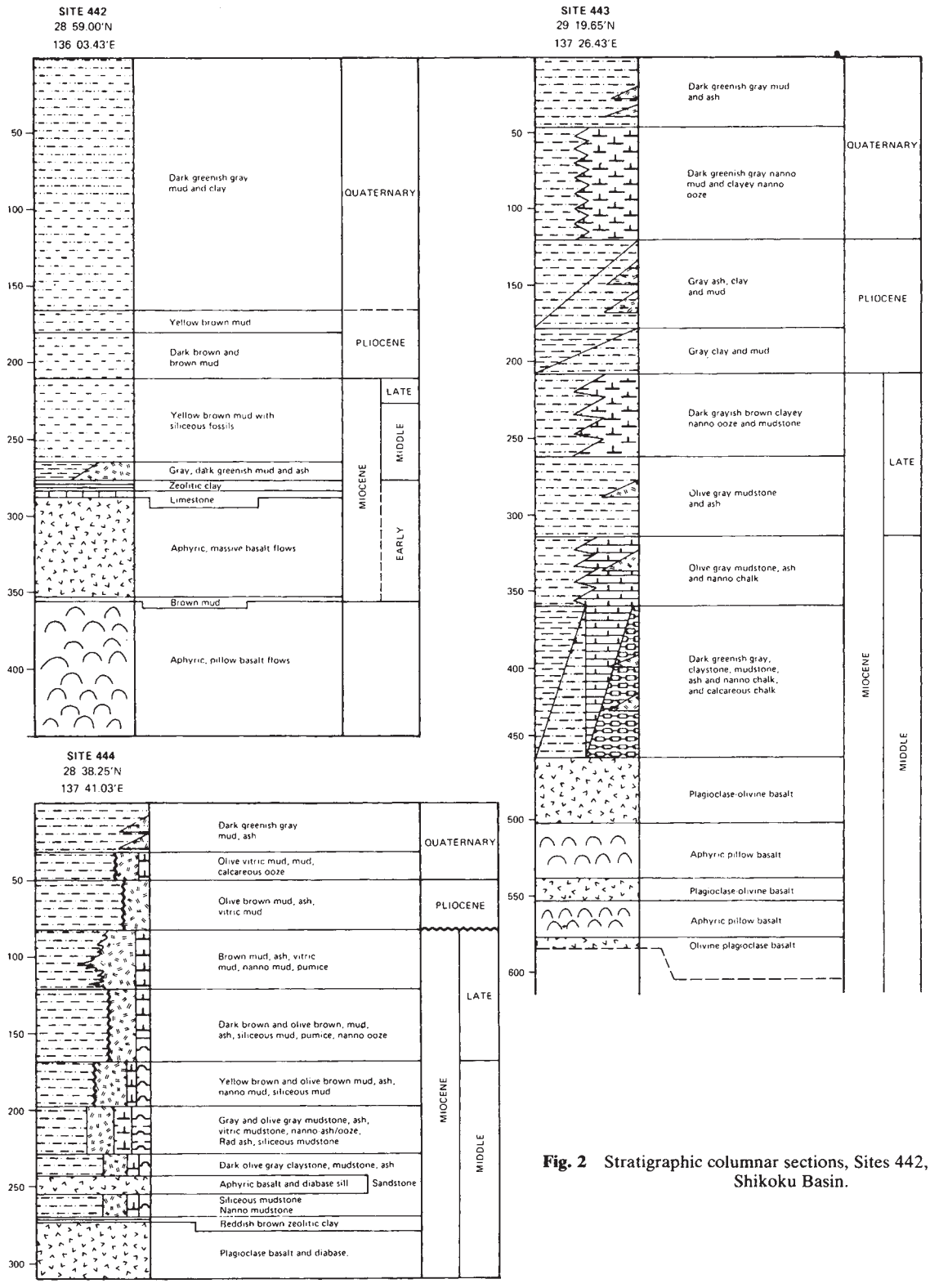

Fig. 2 Stratigraphic columnar sections, Sites 442, 443, and 444, Shikoku Basin.

It is reasonable to assume that the pillow basalts found at Sites 442 and 443 formed where sediment cover was relatively thin and unconsolidated or totally absent. Such an area could exist at or near an active-spreading ridge. Clearly, the massive basalt intrusives and sub-sediment flows erupted after sedimentation had occurred at all three sites. Thus, they postdate extrusion of the pillow basalts and, as a consequence of any reasonable spreading rate, must represent widespread off-ridge volcanism in the basin. This later volcanism occurred over the approximate time span from 13 to 15 Myr BP.

Evidence of off-ridge volcanism has not been recognised previously in marginal basins. Such later volcanism in marginal basins may account for the highly asymmetrical topography mapped in the Shikoku Basin and its rugged nature to the east (K. Kobayashi, unpublished map). The presence of a sill intruding sediment at DSDP Site 285 in the South Fiji Basin[10] suggests that off-ridge volcanism may be characteristic of other marginal basins. This volcanism would explain part of the difficulty that has existed in past mapping and interpreting of magnetic anomaly profiles in these basins.

Table 1 Comparison of age of oldest sediments recovered at Sites 442, 443, and 444

Site	Age of oldest sediment (Myr BP)	Magnetic anomaly and age (Myr BP)	Remarks
442	18–21*	6 (19–20)	Agreement
443	14–15	6A (21–22)	Variance
444	14–15	6A (21–22)	Variance

Magnetic anomaly ages determined by surveys compiled by Kobayashi and Isezaki[6]. Magnetic time scale after LaBreque *et al.*[9].

* This age comes from a sediment interbedded between a massive basalt above it and a pillow basalt below. The age of the sediment immediately above the massive basalt is 15–17 Myr BP.

Magnetic anomaly mapping in the Shikoku Basin[2,5,6,11] indicate the anomalies to be linear and aligned parallel to basin elongation. These anomalies were interpreted previously to be either symmetrical with respect to an extinct spreading centre[2,5,6] or a single-limb spreading process[2]. The minimum basement ages are at variance with the symmetrical model but agree with a single-limb spreading model. Off-ridge volcanism, however, has obscured contact relationships between sediments and oceanic crust that may have formed originally at a spreading centre or a ridge. The ages of the oldest sediment obtained at Sites 443 and 444 may, therefore, not be the true basement age. Thus, we conclude that the age of the oldest sediment recovered is ambiguous in terms of testing models proposed for the style of spreading in the Shikoku Basin. Depending on interpretation, our data could fit any of a number of spreading models for the basin including symmetrical, asymmetrical, or single-limb spreading. Note also that our findings indicate that the age of the ridge (or spreading centre) volcanism at Site 442, the off-ridge volcanism, and the magnetic anomaly mapping[2,5,6,11] prove that the ocean crust of the Shikoku Basin was younger than the Iwo Jima Ridge and Bonin Island Arc[12], which is presently active. Furthermore, the off-ridge volcanism documented here may be of regional significance because it seems to be synchronous with magmatic activity in the south-west Japan arc south of the volcanic front and close to the present position of the Nankai Trough subduction zone[13,14].

The Deep Sea Drilling Project is supported by the NSF and administered by the Scripps Institution of Oceanography. We thank M. N. A. Peterson, D. G. Moore and the project staff for assistance.

G. DEV. KLEIN
University of Illinois at Urbana-Champaign, Urbana, Illinois

K. KOBAYASHI
University of Tokyo, Tokyo, Japan

H. CHAMLEY
Centre Universitaire de Luminy, Marseille, France

D. M. CURTIS
Shell Development Co., Houston, Texas

H. J. B. DICK
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

D. J. ECHOLS
Washington University, St Louis, Missouri

D. M. FOUNTAIN
University of Montana, Missoula, Montana

H. KINOSHITA
Chiba University, Chiba, Japan

N. G. MARSH
University of Birmingham, Birmingham, UK

A. MIZUNO
Geological Survey of Japan, Kawasaki, Japan

G. V. NISTERENKO
USSR Academy of Sciences, Moscow, USSR

H. OKADA
Yamagata University, Yamagata, Japan

J. R. SLOAN
University of California, Davis, California

D. M. WAPLES
Colorado School of Mines, Golden, Colorado

S. M. WHITE
Scripps Institution of Oceanography, La Jolla, California

Received 9 March; accepted 10 April 1978.

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Holocene eustatic sea level change

RELATIVE sea level changes result from eustatic variations in water level and vertical movements of the land, from tectonic or isostatic causes. If these changes are to be properly understood, it is important to define the eustatic component. Devoy[1] has presented a relative Holocene sea level curve for the Thames Estuary, an area of known downwarping throughout the Quaternary and earlier time. If predictions of future relative sea levels in South-east England are to be made, the contributions of the worldwide post-glacial rise of sea level and of regional subsidence in the Flemish Bight have to be evaluated. One approach to the problem is to compare results from areas of tectonic instability with those from more stable areas. In the British Isles, the only area which can be regarded as stable is in the south and west, as far removed as possible, on the one hand, from the subsidence of the south-east, and on the other hand from isostatic rebound from ice-loading centred in North-west Scotland. Although theoretical objections to long-term stability, even in this region, can be adduced[2], the geomorphological evidence in South-west England suggests that, at least in the Holocene and later Pleistocene, the area can be regarded as stable[3]. We presented in 1973 (ref. 4) a sea level curve based on detailed work in the Bristol Channel, which we regarded, in the absence of any evidence of Holocene isostatic and tectonic activity, as representative of the eustatic sea level rise. In view of evidence of continuing isostatic rebound in South-west Scotland[5], studies were subsequently initiated in Cardigan Bay, particularly in the neighbourhood of Aberystwyth and Borth, to try to circumscribe the areas of stability and those of continuing recovery from ice-loading. The results are compared here with our previous ones.

The same criteria were applied to these studies as for the Bristol Channel studies: first, all samples were related to a

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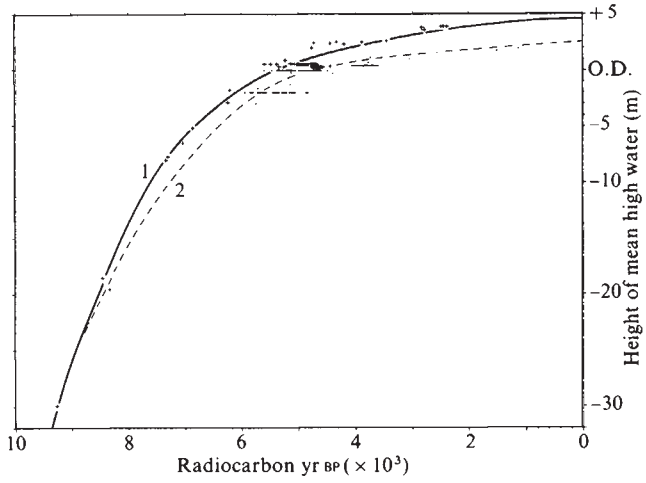


Fig. 1 Sea level curves from the Bristol Channel (curve 1) and Cardigan Bay (curve 2). Curve 1 is plotted from 65 radiocarbon dates (crosses). Curve 2 is plotted from 142 radiocarbon dates (dots). Closely spaced sequences of dates are from dendrochronological calibration series. Standard deviation bars are omitted to avoid confusion. 1σ is typically ±60 yr, the highest is ±160. Mean high water is 2 m higher in the Bristol Channel than in Cardigan Bay, and when this correction is applied, no significant difference can be seen between curves 1 and 2.

water table in turn linked to sea level, using evidence from pollen, macroscopic plant remains, diatoms and foraminifera. No ombrogenous peats were used. Second, trees *in situ*, or peats, were dated. No salt marsh peaty clays were used, as these were found to occur over a large and unpredictable height range. Third, almost all the samples in each of the two areas were from closely spaced sites, where the overall stratigraphy and vertical and horizontal successions are understood. Finally, sites where an appreciable and unknown amount of consolidation has occurred, were avoided.

Figure 1 shows two separate curves from the Bristol Channel (1) and from Cardigan Bay (2). Curve (1) does not differ significantly from the previously published Bristol Channel curve[4,6] although it includes more radiocarbon dates.

In both areas the stratigraphy and the shape of the curve are quite clear for the earlier and later periods. It is only in the period 4,000–5,000 yr BP that the sequence of events is, at first sight, unclear. It seems that, before this period, sea level rise was so rapid, and the position of the coast, consequently, so transient, that only during brief and widely separated intervals could peat formation occur. Each date from this period, therefore, represents an unambiguous sea level. After 4,000 yr BP, on the other hand, sedimentation was in equilibrium with the slow rate of sea level rise and the ^{14}C dates, therefore, accurately reflect the rate of this rise.

Between 5,500 and 4,000 yr BP the situation is more confused. The extensive growth and subsequent inundation of submerged forests at this time shows that the coastline was fluctuating. These fluctuations could result from a stand or fall in sea level or from other causes. Fortunately, the existence of the submerged forests enables us to answer the question they pose. Dendrochronological investigations of the relative ages of trees at various altitudes in the submerged forests in Bridgwater Bay, at Borth and elsewhere[7] show that sea level rise was, during the lifetime of a tree, negligible as an agent of coastal inundation when compared with exceptional storms. It was, however, still too rapid for a state of equilibrium to be reached. As a result of local changes in sedimentation, trees were able to establish themselves on alluvial surfaces and to grow there for periods measured in centuries, until inundated by an event of magnitude comparable to that of the East Coast floods of 1953. A new and higher water table was thus established, the trees being preserved, and subsequently surrounded by fen or carr peat, building up to this new water table. At some stage in this process the trees were usually uprooted or broken off, so that both stumps and fallen trees are found. The obvious interpretation of a series of ^{14}C dates through such a section would be that it represented a fairly rapid rise in sea level, whereas all that is actually being measured is the rate of peat accumulation. Only by dendrochronological correlation of the trees throughout a submerged forest, when the relative ages are known to within one year, can the true course of events be seen. It is apparent that an instantaneous rise in the water table, as described, cannot be plotted as a 'wiggle' on the sea level curve. The only rational course is to plot the intervals between such events as a smooth curve, whilst noting that the transgression was, in fact, largely accomplished by discrete events.

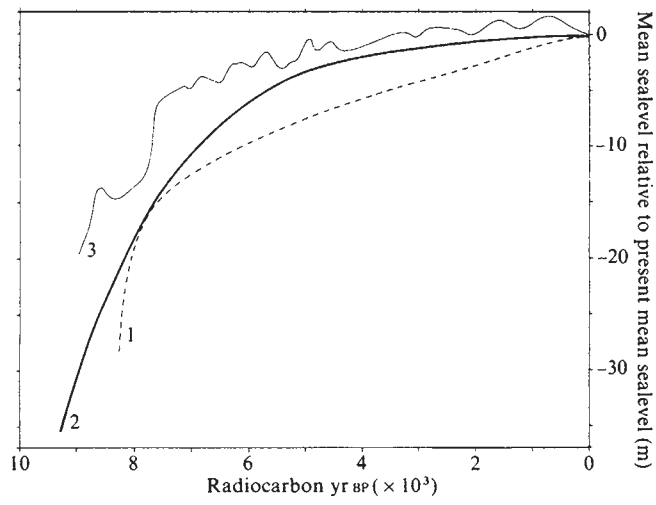
There seems little reason to invoke oscillations in sea level (of magnitude less than that of storm surge events) to account for any other transgressive or regressive sequences observed. The curves given here are drawn on this basis.

In addition to the ^{14}C dates shown in Fig. 1, many trees were dated dendrochronologically, in calendar years, relative to those with radiocarbon dates. These dates are not plotted, as it would be necessary to convert calendar years to radiocarbon years, but they have influenced the position of the curve. These tree-ring studies have shown that it is not possible to relate minor differences in the altitudes of individual trees to minor fluctuations in sea level.

The agreement between the Bristol Channel and Cardigan Bay curves suggests two important conclusions. First, the two areas must be regarded as comparable in terms of isostatic readjustment. They must be either outside the region of rebound from Devensian ice-loading, or within a zone where this adjustment was completed very early.

Second, as the two areas are separated by some 180 km, there is a strong probability that both are within an area of long-term crustal stability. It seems unlikely that a block of such a size could move vertically with no tilt. Such long-term stability must, of course, be distinguished from the dynamic equilibrium between subsidence and isostatic uplift which gives rise to an apparently stable area in the vicinity of the Humber.

Fig. 2 Sea level curves compared. Curve 2 is plotted from all the Bristol Channel and Cardigan Bay dates in Fig. 1, corrected for the difference in mean high water levels. Curve 1 is from the Thames Estuary[1]. Curve 3 is from the Lancashire Coast[8]. The relative positions of the three curves are corrected for differences in tidal range (see text) and are plotted on a vertical axis representing changes of sea level relative to a common present-day zero.



In Fig. 2, curve 2 is drawn from all the dates shown in Fig. 1, adjusted to compensate for the difference in tidal range, by raising the Cardigan Bay dates by 2 m, relative to the Bristol Channel dates. This represents the difference between the height of mean high water at the two places.

Curve 1 is Devoy's[1] smoothed curve for the Thames Estuary and curve 3 is Tooley's[8] curve from the Lancashire Coast. Both these curves are also adjusted to compensate for tidal differences by raising curve 1 by 1.8 m and curve 2 by 1.4 m relative to the Bristol Channel heights.

As these curves show relative sea levels, they equally well represent changes in mean sea level, mean high water spring tide, and so on. Assuming that curve 2 represents eustatic sea level rise, the relative vertical displacements of curves 1 and 3 indicate the amount of subsidence and uplift of the land, respectively, after any given date.

The overall trends seem to be as expected, the north-west showing a decreasing rate of isostatic uplift, and the south-east a more or less uniform rate of subsidence.

However, the coincidence of curves 1 and 2 at 8,000 yr BP is not easily explained. This would indicate subsidence before this date followed quickly by rapid uplift and then by renewed subsidence. Such a pattern of events seems unlikely, and other factors may be responsible for the shape of curve 1.

In the south-east, a uniform rate of subsidence of some 4 m in the past 4,000 yr is indicated, together with a sea level rise of 2.3 m in the same period. At present, it seems that subsidence is responsible for almost all the relative sea level rise in the south-east.

In the north-west the extent of isostatic uplift has been 10 m since 9,000 yr BP, 6 m since 7,000 yr BP and 2 m since 5,000 yr BP. It seems that uplift is still proceeding, though at a much diminished rate.

It is interesting that the Cardigan Bay/Bristol Channel curve is almost perfectly exponential in form. This perhaps suggests a return to equilibrium following a sudden change in the glacio-eustatic balance. 10,000 BP is suggested, both by $^{16}O/^{18}O$ measurements[9] and by studies of Coleoptera[10] as the date of the sudden ending of a prolonged cold period. This may have been the start of the exponential rise of sea level which has continued until the present.

If the curve is extrapolated back to 10,000 yr BP (the earliest ^{14}C date is 9,260 yr BP) a mean high water level of −50 m is indicated at that date. It may be that a pause in sea level rise ending at 10,000 yr BP was responsible for the formation of the submerged cliff line around the south-west peninsula at about −45 m O.D. (ref. 11). The earlier course of eustatic rise from ≃−100 m O.D. is still more speculative.

We thank Dr Murdoch Baxter for many of the radiocarbon dates used.

C. KIDSON

A. HEYWORTH

Department of Geography,
The University College of Wales,
Aberystwyth, UK

Received 28 March; accepted 20 April 1978.

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Stable carbon isotopes in human tissues

ON studying the distribution of ^{14}C from nuclear weapon tests in a wide range of human tissues, it was observed that significant modification of $^{14}C/^{12}C$ isotopic ratio had occurred, largely as a function of variable carbon renewal rates[1,2]. As a parallel exercise, $^{13}C/^{12}C$ isotope ratios have been assayed, ^{13}C and ^{12}C being the nonradioactive isotopes of carbon which are assumed to be unaffected by nuclear testing. In principle, however, it is feasible that the differing biochemistries and carbon renewal rates of various body tissues could induce measurable stable isotope ratio changes (fractionation) as a result of the discrepancy in nuclear mass between ^{12}C and ^{13}C. This inequality leads to a difference in basic thermodynamic properties manifested as zero point energy, diffusion coefficients and kinetics of reaction for ^{12}C- and ^{13}C-substituted molecules. Thus, in reactions at equilibrium or those kinetically controlled, preferential selection of either the light or heavy isotope, and hence $^{13}C/^{12}C$ ratio change, may occur. We report here the results of over 80 measurements on human tissue samples which have increased the data available on natural baseline isotope ratios. This is particularly relevant because of the increasing use of stable, rather than radioactive, tracers in metabolic studies.

Stable isotope ratios are normally expressed as an enrichment or depletion of ^{13}C in a sample by the parameter $\delta^{13}C$ defined as

$$\delta^{13}C = \left(\frac{R_S}{R_{STD}} - 1\right) \times 1{,}000‰$$

where R_S and R_{STD} are the $^{13}C/^{12}C$ ratios of the unknown and standard, respectively, and $\delta^{13}C$ is the per mille deviation of the sample $^{13}C/^{12}C$ ratio from that of a standard material which is generally the primary international PDB standard (*Belimnitella americana*). On this scale, naturally occurring values of $\delta^{13}C$, precise to within ±0.1‰, normally lie within the range +5‰ to −30‰. Some examples are: atmospheric CO_2, −7 to −10‰; carbonate minerals, +4 to −4‰; land plants, −24 to −29‰; marine plants, −8 to −17‰ (ref. 3). These data show that a major fractionation step is involved in the fixation of atmospheric CO_2 to form plant tissue. The relevant question, then, is whether similar ratio changes occur during human metabolic processes.

Table 1 $\delta^{13}C$ variations in tissue from a single individual

Organ	$\delta^{13}C$(=)
Spleen	−22.16
Lung	−22.38
Thymus	−25.59
Liver	−22.72
Brain	−21.05
Pancreas	−25.25
Thyroid	−22.74
Heart	−22.79
Testes	−22.25
Muscle	−23.56
Kidney	−24.00

Table 2 $\delta^{13}C$ in body fluids

Sample	$\delta^{13}C$(=)
Metabolic CO_2	−22.53
Metabolic CO_2	−23.61
Metabolic CO_2	−23.59
Metabolic CO_2	−22.74
Blood plasma	−18.73
Blood	−18.22

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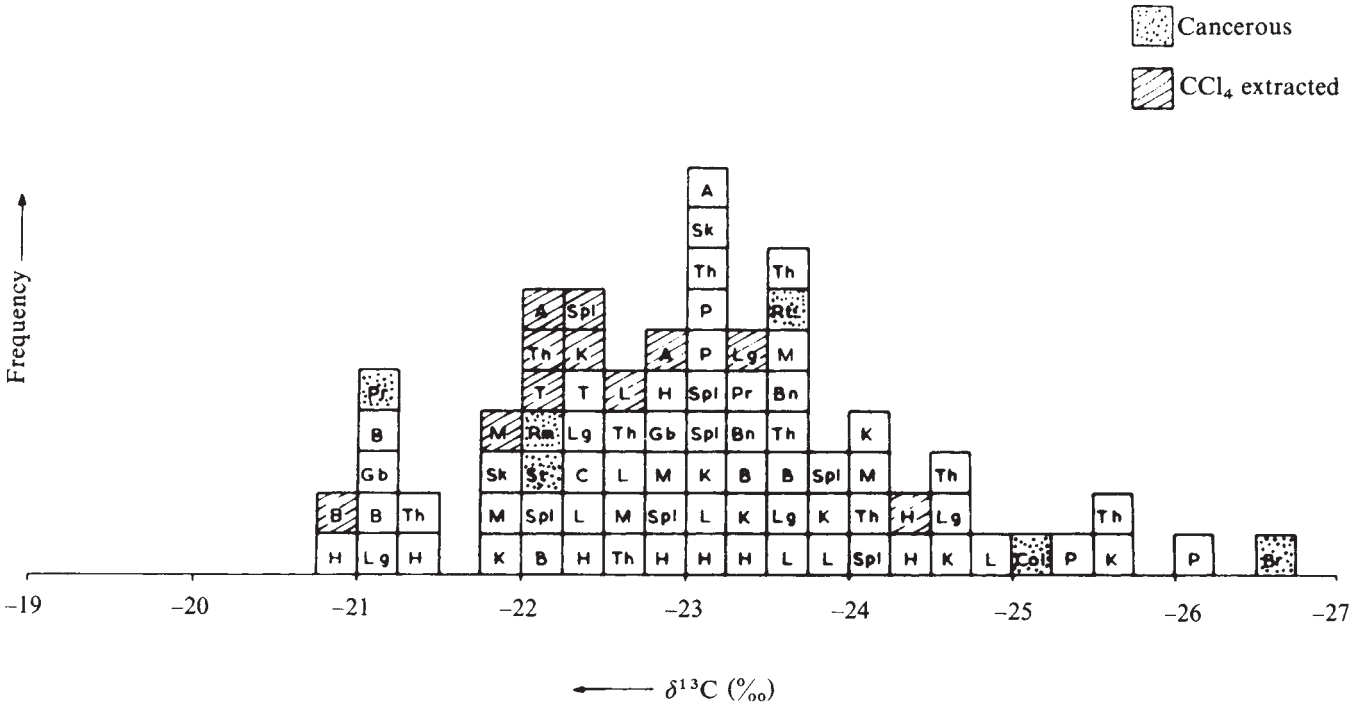

Fig. 1 Histogram of $\delta^{13}C$ data for organic carbon in healthy and diseased human tissues. A, adrenals; B, brain; Bn, bone; Br, breast; C, collagen; Col, colon; Gb, gall-bladder; H, heart; K, kidney; L, liver; Lg, lungs; M, muscle; P, pancreas; Pr, prostate; Rm, rectal muscle; Rt, rectal tumour; Sk, skin; Spl, spleen; St, stomach; T, testes; Th, thyroid.

Tissue samples (about 10 g) were collected and frozen following post-mortem examinations. These were then homogenised and freeze-dried before conversion to CO_2 gas for mass spectrometric analysis on a VG Micromass 602 double collector instrument using an all-metal McKinney type changeover valve and a metal inlet system. Sufficient sample to provide 10–20 cm^3 (STP) of CO_2 was combusted in a flow system similar to that of Lerman[4] but with the addition of an MnO_2 furnace to remove contaminating NO_2. Carbonate samples were hydrolysed with 100% H_3PO_4 at 25°C and metabolic CO_2 was collected by passing exhaled air through dry ice/acetone traps (to remove H_2O) into a liquid N_2 trap. Blood samples (40 cm^3) were stored under liquid paraffin and the CO_2 stripped out within one hour of collection using H_3PO_4 and flushing with CO_2–free N_2 in a similar way to that used for oceanographic samples[5]. Standard combustions indicated an overall efficiency of 100%. This is an important prerequisite, as variations in $\delta^{13}C$ resulting from experimental errors must be minimised. Replicate analysis of the same CO_2 sample indicated a precision of ±0.03‰ and replicate analyses of pure graphite including combustion indicated a larger system error of ±0.2‰. Analyses of NBS 21 (graphite) standard gave a mean value of −27.85 ± 0.10‰, compared to −27.79‰ (ref. 6) and −28.19‰ (ref. 7) reported by Craig and Mook respectively.

The $\delta^{13}C$ values of 76 healthy tissue samples and six samples of cancerous tissue are shown as a histogram in Fig. 1. The variations of $\delta^{13}C$ in tissue from a single individual are shown in Table 1 and for body fluids in Table 2.

Figure 1 shows that $\delta^{13}C$ values for normal tissues fall between about −21‰ and −26‰ with a maximum frequency at −23‰. The degree of scatter is well outside the limits of experimental error and indicates a significant variability in the isotope ratios of human tissues. There is, however, no apparent trend with tissue type, age or sex; nor does cancerous tissue show any significant difference from healthy tissue. Samples extracted with CCl_4 tend to fall on the less negative side of the maximum. This implies, perhaps, that the isotope ratios of component biochemical fractions such as fats and proteins may be less variable than those of whole tissues of variable composition. Samples of metabolic CO_2 fall close to the distribution maximum.

Bone carbonate is enriched in ^{13}C by about 10‰ relative to soft body tissue, and total blood and blood plasma yield a mean value of −18.50‰ so that fractionation between $HCO_{3_{blood}}^-$ and $CO_{3_{bone}}^-$ is 5.34‰, and bicarbonate is enriched by about 4.5‰ relative to metabolic CO_2. These fractionations are broadly consistent with the corresponding fractions found in the calcite and aragonite of living shells (for which the CO_3^{2-} of shell is enriched by some 3–8‰ relative to the HCO_3^- of seawater).

A secondary observation relates to the use of stable isotope tracers in medical studies—that a final ^{13}C-enrichment of ~10‰ is the minimum required for significant artificial alteration. Any lesser change in whole tissue ratios could be attributed to natural fractionation effects.

The results presented here add significantly to the baseline data on natural isotope ratios in humans. They imply a measurable degree of fractionation in metabolic processes but show a random distribution for whole tissue $\delta^{13}C$ values. There is no systematic fractionation apparent during replication of cancer cells, as may have been expected through kinetically induced selection processes. Fractionation in formation of bone carbonate parallels accretion of shell carbonates in nature and reflects 'organic guidance of an inorganic process'. Minimum significant enrichments in stable isotope tracer studies are defined.

This work was supported by the MRC. We thank W. A. Harland and J. S. Dunn for providing documented post-mortem samples.

T. D. B. LYON*
M. S. BAXTER

Chemistry Department,
University of Glasgow,
Glasgow, UK

Received 10 April; accepted 27 April 1978.

* Present address: Department of Clinical Physics and Bio-Engineering, Greater Glasgow Health Board, 9 West Graham Street, Glasgow UK.

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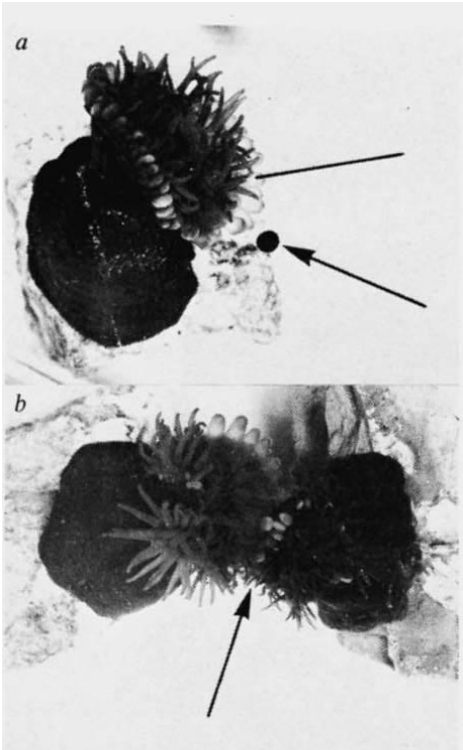
Size-dependent dominance hierarchy in the anemone *Actinia equina*

THERE are many examples of aggression among anthozoan coelenterates: corals show interspecific interaction in which ranking may be obvious[1], and inter-clonal aggression occurs in the anemone *Anthopleura elegantissima*[2]. Aggressive behaviour is also exhibited by some solitary anemones, such as *Actinia equina* L. Both these species possess special structures bearing batteries of nematocysts, the acrorhagi which are used solely for offence[3]. We report here that there is a hierarchy of aggression, which is based on size, in *A. equina*.

We used individuals varying in colour from red to brown; no differences in behaviour were apparent within this colour range. Green *A. equina* have an aggressive sequence which differs qualitatively from that described in Fig. 1, and are not considered here. Anemones were collected from Falmouth, Cornwall and maintained in circulating seawater at 10°C for 6 months before experimentation, in a 12-h light and 12-h darkness regime; they were fed with *Mytilus edulis* every 7 d. Experiments were carried out in the anemone's daytime, but not during the 48 h following feeding. Individuals were retested at intervals of >4 h. In each experiment, two anemones, each firmly attached to a small rock or stone, were transferred to a vessel containing 1 litre of aerated seawater at 10°C. After a recovery period of 10 min, the anemones were slowly brought towards one another until contact was established by one or two pairs of tentacle tips. Only fully expanded individuals were used. This minimal initial contact which lasts 1–10 s, was sufficient in most encounters (>95%) to elicit an aggressive sequence from at least one individual, which in some cases did not run to completion. This is comprised of discrete stages[4] (Fig. 1), the whole sequence lasting between 7 and 10 min. Normally a 'winner' and 'loser' could be distinguished: the winner remained open and active, and the loser partially or totally closed, and sometimes detached from the substratum. In some cases, however, the second individual retaliated, and occasionally the initial aggressor aggressed for a second time. If, after retaliation, both anemones remained open, the contest was deemed a stalemate.

Fig. 1 Aggressive behaviour of *Actinia equina*. *a*, Column bending away from point of contact (arrow) with opponent, and acrorhagial inflation (pointer); *b*, acrorhagial contact (arrow), after column bending and extension towards second individual (to right), resulting in infliction of surface damage to opponent.



Using the results accruing from many encounters, we have constructed two hierarchies (Fig. 2) based on 'aggress first' (initial aggressor) and 'win'. A comparison of the ranking orders between these (Table 1) shows that the initial aggressor almost invariably 'won' the encounter. Specific encounters which were repeated did not yield contradictory outcomes. We assessed the importance of relative size (Fig. 2*a*) by examining the correlation between expected first aggressor and both observed aggressor and 'winner'. There is a good correlation in both cases (aggressor, $P<0.005$, $n=26$; 'winner', $P<0.006$, $n=16$).

Table 1 Ranking order assigned to anemones

Anemone no.	Rank: Aggress first	Rank: 'Win'
3	1	2
6	2	2
11	3.5	6
20	3.5	2
4	5.5	4
16	5.5	10
2	7	6
7	8.5	10
13	8.5	10
21	10	6
26	11.5	10
24	11.5	10

Rank correlation between hierarchies, $r=0.797$, $P<0.005$, $n=12$.

Aggressive behaviour could be evoked by lightly touching one or two tentacle tips of an intact anemone with the tip of an amputated tentacle from another individual. Once aggression was evoked, the sequence continued in the absence of further presentation of the stimulus, suggesting, in common with much invertebrate behaviour[5], that a motor programme underlies this sequence. The number of contacts sufficient to initiate aggression in the ranked anemones using this method ranged from 2 to 16 (mean 6.3); the amputated tentacle was presented for 0.5 s at 1-min intervals. Preliminary data indicate a good correlation between the number of contacts and the absolute size of the anemone, being greatest for small anemones.

Our results indicate a size-related difference in aggressive response. Responsiveness is enhanced in larger anemones so that they aggress sooner, starting soon after initial contact. Several factors could account for the observed difference in threshold. These include alterations in the integrative properties of the nerve net and neuroid systems[6,7], perhaps mediated by a change in spontaneity[8]. The involvement of more than one conduction system is suggested by a comparison with the shell climbing behaviour of *Calliactis parasitica*[9] which includes several orientated behavioural components similar to those used during aggression. We suggest that as with other complex anthozoan behaviours[10,11], a surface-bound substance may prove to be the potent stimulus eliciting aggression.

The situation imposed on pairs of anemones in our experiments was somewhat artificial in that both anemones were attached firmly to the substratum. We presume that one individual would normally be mobile, and it will be important to establish whether the 'resident' anemone has an 'advantage', as is frequently the case in aggressive interactions[12].

This branching and overlapping dominance hierarchy is the first of this kind to be demonstrated in a lower invertebrate, although relative size is clearly an important determinant in vertebrate and arthropod aggression[13,14], smaller individuals commonly being submissive, thus minimising severe damage

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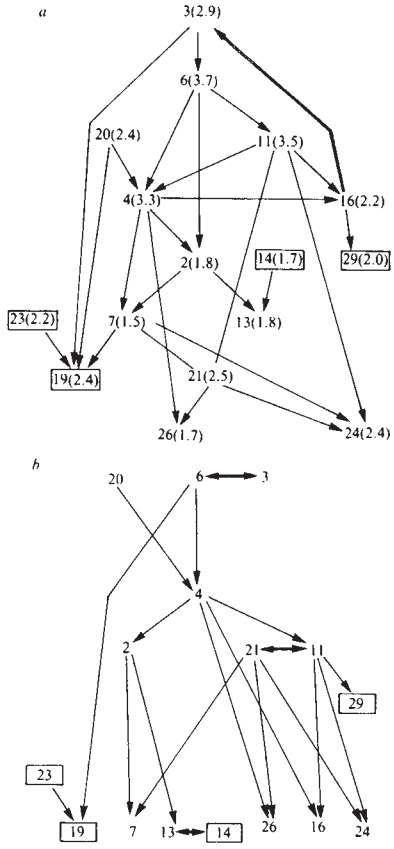

Fig. 2 Ranking order of anemones (numbered) based on (*a*) aggress first and (*b*) 'win'; □, anemones whose rank cannot be adequately assessed with respect to both hierarchies; ↔ stalemate following retalliation; ⇒ only 'ring' demonstrated. Figures (cm) in brackets refer to size (pedal disk diameter); discrepancy in number of encounters between *a* and *b* accounted for by sequences which did not run to completion.

during encounters. The limited mobility of *A. equina* has presumably contributed greatly to the development of such a system. The establishment of the 'size-convention' lies not in behavioural interaction involving a 'trial of strength', but apparently in size-dependent modification in responsiveness which allows larger anemones to aggress before their smaller opponents, and subsequently to 'win' encounters.

We thank D. L. J. Quicke for discussions and help, and Professor P. N. R. Usherwood for helpful criticism.

R. C. BRACE
JANIS PAVEY

Department of Zoology,
University of Nottingham,
University Park,
Nottingham, UK

Received 9 February; accepted 21 April 1978.

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Chromosome transfer in dikaryons of a smut fungus

THE haploid sporidial stage of the anther smut fungus, *Ustilago violacea*, consists of yeast-like uninucleate cells of two mating types, a_1 and a_2. When complementary auxotrophs of opposite mating type are conjugated and plated on minimal medium (MM), several types of prototrophic colonies grow up, the most distinctive of which are the translucent colonies of diploids, heterozygous for mating type and formed by karyogamy. These appear with a frequency of about 3 per 10^4 conjugated cells[1] and have been exploited for mitotic mapping in this species[2]. The other types of prototrophs seem to carry only one mating-type allele and form opaque colonies. These prototrophs form with a frequency of about 1 per 10^3 conjugated cells, and we show here that they arise from either conjugant after a number of chromosomes have been gained from the other conjugant. We also show that most of them are not conventional aneuploids, but that they have the extra chromosomes sequestered in such a way that they are lost or gained as an entity. The frequency of these segregants and their stability suggest that this process may play a hitherto unsuspected part in the origin of new physiological races in this species and perhaps also in other fungi.

The prototrophs occur about 10^3 times more frequently than they do in unmated cultures and therefore do not arise by spontaneous mutation. The results of a typical experiment are outlined in Fig. 1. A yellow (y) a_2 strain, carrying the linked genes lys_3 and cit_1 (located 40 map units apart[2] and equidistant from the centromere (unpublished data)) was conjugated with a pink (y^+) a_1 strain carrying the unlinked markers arg_1 and his_4 (ref. 2). The conjugated cells were plated on MM and on MM supplemented with one of the requirements of the stocks. Yellow prototrophs were as frequent on MM as on the supplemented MM plates and were all a_2 in genotype. Moreover, all the yellow colonies that grew up on MM + lysine or MM + arginine had acquired both lys_3^+ and cit_1^+, even though one of these markers was not selected for. Thus the two alleles 40 map units apart were always acquired simultaneously, presumably by transfer of a whole chromosome.

On the other hand, pink prototrophs were about 10 times less frequent on MM or MM + lysine than on MM + arginine or histidine. On MM + arginine or histidine about 85–90% of the pink a_1 colonies were $arg_1\ his_4^+$; only 10–15% had acquired both arg_1^+ and his_4^+. On all media, some pink prototrophic strains were found that were a_2 in mating type. These pink colonies occasionally produced yellow sectors and were thus heterozygous y/y^+. They therefore seem to be derived from yellow a_2 strains that had acquired a y^+ chromosome in addition to the $lys^+\ cit^+$ chromosome. Clearly, in the absence of selection, there is only a relatively small chance (about 10%) of two chromosomes being acquired together. Studies with many other combinations of linked and unlinked markers have confirmed that linked genes are always co-acquired, whereas unlinked genes are co-acquired with a frequency of 10–20%. As only $lys^+\ cit^+$ were recovered from the yellow a_2 parent, crossing over cannot be occurring with any appreciable frequency.

The chromosome number of *U. violacea* is not known but has been estimated to be about 20, of which 12 can be identified with markers[2]. The frequency of about 10% co-acquisition thus suggests that the number of chromosomes transferred is small, probably less than 5. The cells carrying these extra chromosomes have a reduced growth rate, but are relatively stable, generating the original parental genotype at frequencies of less than 1×10^{-3}. Thus the pink a_2 prototrophs of Fig. 1 give rise to stable, vigorous y, lys_3, cit_1, a_2 segregants. The segregation of parental genotypes and the lack of any mitotic recombination indicate that the extra chromosomes are kept separate and do not form a conventional aneuploid. It also

0028–0836/78/0273—0753$01.00

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Conjugated pair of cells

Selection medium	Genotypes recovered from a_2 conjugant	No. of colonies per plate*	Frequency†	Genotypes recovered from a_1 conjugant	No. of colonies per plate*	Frequency†
Minimal medium (MM)	y lys3 cit1 + + a2 / + +	89	5.2			
				+ + + arg1 his4 a1 / + +	7	0.41
	y lys3 cit1 + + a2 / + + +	10	0.59			
MM + arginine	y lys3 cit1 + + a2 / + +	96	5.6	+ + + arg1 his4 a1 / +	64	3.8
	y lys3 cit1 + + a2 / + + +	7	0.41	+ + + arg1 his4 a1 / + +	8	0.47
MM + histidine	y lys3 cit1 + + a2 / + +	80	4.7	+ + + arg1 his4 a1 / +	96	5.6
	y lys3 cit1 + + a2 / + + +	9	0.53	+ + + arg1 his4 a1 / + +	9	0.53
MM + lysine	y lys3 cit1 + + a2 / + +	88	5.2			
				+ + + arg1 his4 a1 / + +	9	0.53
	y lys3 cit1 + + a2 / + + +	9	0.53			

Fig. 1 Origin of prototrophic recombinants from conjugated auxotrophic cells of *U. violacea*. Strains of the genotypes shown[2] were conjugated and 1.7×10^5 conjugated cells were plated on each of four plates of each of the selection media listed. After 14 d, the colonies that grew up (other than a_1a_2 diploids) were counted and tested for genotype by replica plating. The genotypes recovered, together with their frequency per 10^4 conjugants, are given in the figure. Prototrophic alleles acquired by one conjugant from the other conjugant are shown in bold type. *, Mean of four plates. †, Frequency of colony per 10^4 conjugated cells (= mean no. colonies $\times 10^4/1.7\times10^5$).

seems that the acquired chromosomes are generally lost as a group.

To confirm these findings, a $y.a_1$ strain carrying the recessive markers his_1, uvs_3 (ultraviolet sensitive) and cxr_1 (cycloheximide resistant) was conjugated with a pink a_2 strain carrying the lys_2 and $inos_1$ (inositol requiring) alleles (Fig. 2). All of the yellow prototrophs that arose on MM, had acquired the cxr_1^+ allele together with the selected marker (his_1^+), suggesting that these two alleles were linked. About 10% had also acquired the uvs_3^+ allele, which is on a separate chromosome. Six of these yellow a_1 colonies that had acquired his_1^+, cxr_1^+ and uvs_3^+ were replica plated to complete medium containing cycloheximide. The majority of cells express cxr^+ and therefore cannot grow on this medium. At low frequencies, however (about 10^{-3}–10^{-4}), cells arose which expressed the recessive cxr_1 allele and therefore formed sectors. These sectors were always found to have reverted to a completely parental genotype, that is, they had lost the three dominant alleles and were again $y.his_1\ uvs_3\ cxr_1\ a_1$. Similar results have been obtained using strains carrying other markers and a different *cxr* gene.

The acquired chromosomes are thus additional to, rather than replacements of, their homologues in the recipient and they do not mix randomly with the recipient genome to form a conventional aneuploid, but are organised separately such that they can be lost or gained as a whole. The simplest explanation is that they form a micronucleus (or a multichromosomal plasmid), but studies with acridine orange fluorescence have not provided clear support for this. Although in occasional prototrophic segregants many of the cells have a normal nucleus and a micronucleus, in most of the others only 1–5% of cells have a discernible micronucleus. Even this low frequency is significant, as binucleate cells of any kind are not seen in normal haploid populations. The low frequency of ex-conjugant prototrophic cells with visible micronuclei does not seem to indicate a frequent breakdown to truly haploid cells, as plating experiments show that nearly all the cells still express the acquired markers. Thus the micronucleus may not normally be visible under the light microscope because of its extremely small size, or because it may be adpressed closely against the main nucleus. Alternatively, there may be only one nucleus with the acquired chromosomes organised separately within it. In this case, the few cells with micronuclei would be a result of breakdown of this organisation. Models of nuclear division in fungi have been proposed which would allow for this kind of internal organisation in the fungal nucleus[3]. Electron microscopic observations are planned to investigate these possibilities further.

It does not seem probable that the prototrophic segregants derive from a transient diploid nucleus, as this would not explain the exclusive breakdown to parental genotypes, and the concomitant absence of recombination. Similarly, the absence of crossing over argues against any pseudomeiotic process. Instead, it seems likely that an unstable dikaryon occurs in which there is nuclear breakdown of either nucleus, followed by establishment of many different lines carrying different acquired chromosomes. The chance of any selected marker appearing in these lines was about 5 per 10^4 conjugated cells and therefore, with about 20 chromosomes, the total frequency of the process may be as high as 1 per 10^2 conjugations.

Clearly, this frequency is high compared with either parasexual recombination or spontaneous mutation[4,5], and chromosome transfer/acquisition may therefore be very important in nature as a source of genetic variation. Many of the physiological races of smuts, rusts and perhaps other groups of fungi, may have arisen in such a way. Furthermore 'new' races may seem to be generated when previously acquired chromosomes are lost. Thus the common and poorly understood phenomenon of frequent somatic variation in certain supposedly haploid fungi (saltation) may perhaps be due to the loss of extra chromosomes that had been acquired previously. It is also possible that once chromosomes have been acquired, they may be transmitted to other strains at very high frequency in subsequent conjugations.

P. R. Day has reviewed[5] the data on recombination and the origin of new races in groups such as the rusts and smuts and has pointed out the extreme flexibility of "reassociation and recombination of nuclei, chromosomes, genes and cytoplasmic elements accompanying vegetative growth"[5]. The mechanisms put forward to account for some of the recombinant strains arising in *Puccinia graminis tritici*[7], *P. coronata*[8], *Schizophyllum commune*[9] and *Melampsora lini*[10] are varied and somewhat speculative, involving nuclear transfer, chromosome exchange, specific mating-type exchange, and deletions of genes or chromosomes. Some of these observations may be reinterpreted in terms of the acquisition of extra chromosomes, but clearly a thorough investigation of the potential for nuclear rearrangements in multinucleate cells of fungi is needed. The potentialities for exchange and recombination are much greater than was once supposed and account for the great versatility and adaptability of fungi.

I thank Dr J. E. Cummins for his interest and criticism of this work, and Adrian Newton for technical help.

ALAN W. DAY

Department of Plant Sciences,
University of Western Ontario,
London, Ontario, Canada N6A 5B7

Received 28 December 1977; accepted 7 March 1978.

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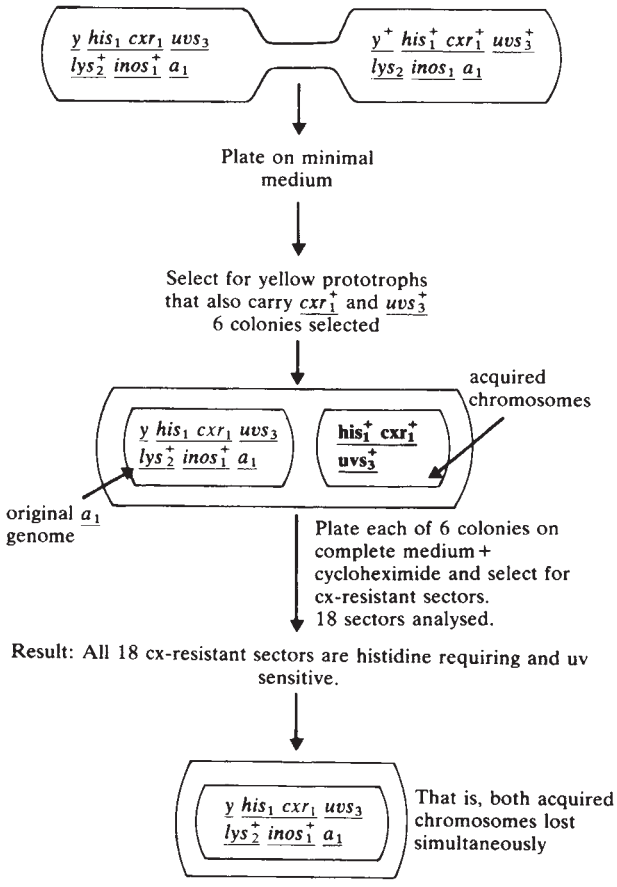
Fig. 2 Simultaneous loss of acquired chromosomes following selection for cycloheximide resistance. Strains of the genotypes shown[2] were conjugated and plated on minimal medium. Yellow prototrophs were selected and tested for ultraviolet and cycloheximide (cx) sensitivity. Six yellow prototrophs that were ultraviolet resistant and cx sensitive were replica-plated to complete medium plus 100 μg ml^{-1} cycloheximide. Eighteen cx-resistant sectors were isolated (three from each of the six yellow prototrophs) and analysed for genotype. Acquired chromosomes are shown in bold type.



Further evidence for a random transition in the cell cycle

SEVERAL proposals have been advanced to explain how cell cycle duration (and hence proliferation rates) is governed[1–3]. It is important to establish which (if any) of these models is correct as they imply differing biological mechanisms of cell-cycle control. The transition probability hypothesis divides the cell cycle into two phases A and B, the transition from A to B occurring at random[3]. As a consequence a graph of the logarithm of the percentage of undivided cells (α_t) against time after mitosis (the α curve) shows two components since α_t will be 100% for times up to the minimum cycle time T_B (the duration of B phase) and decay exponentially thereafter. This model has been criticised on the grounds that other models of the cell cycle can generate similar α curves and on the basis of the α curve it is difficult to tell these models apart[4]. In what follows the exponential nature of the distribution of differences of sister cell cycle times (the β curve) is shown to be a unique property of cell cycle models containing an exponentially distributed phase.

Let the probability of a cell remaining undivided for a time $\geqslant t$ after its birth be $Q(t)$. Then the probability that a cell has already divided is $1-Q(t)$. Hence the probability of a cell dividing in the interval t to $t+\mathrm{d}t$ is

$$-\dot{Q}(t)\,\mathrm{d}t \qquad (1)$$

where $\dot{Q}(t)$ denotes differentiation of $Q(t)$ with respect to time. The probability that another cell remains undivided after a further time T has elapsed is then

$$Q(t+T) \qquad (2)$$

if the probability of a cell remaining undivided at a given cell age is identical for all cells. This simplifying assumption can be relaxed as β curves take the same form whatever sub-grouping of cells is considered but the assumption is introduced as it simplifies the mathematics (data not shown).

The probability $\beta(T)$ that the intermitotic time of two cells will differ by an amount greater or equal to T is given by

$$\beta(T) = -2\int_{t_1}^{t_2} Q(t+T)\dot{Q}(t)\,\mathrm{d}t \qquad (3)$$

where the earliest dividing cell of the pair divides between t_1 and t_2. The factor 2 is introduced since the order in which cells

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of the pair divide is immaterial. According to the transition probability hypothesis no cell divides at $t < T_B$, once T_B is exceeded then

$$Q(t) = \exp(-k(t - T_B)) \quad (4)$$

where $(t - T_B)$ is the time spent in the A state. If all cell pairs with a cycle time $\geq T_B$ are considered then substituting $t_1 = T_B$, $t_2 = \infty$ and $Q(t)$ from equation (4) into equation (3) it may be seen that $\beta(T) = \exp(-kT)$. In other words the β curve will be exponential with the same slope as the α curve providing T_B is the same in the pairs of cells chosen and all cells obey the same kinetic law. In practice the β curve is exponential for pairs of sister cells implying that T_B is the same in sister cells[5-8].

The experiment illustrated in Fig. 1*a* shows α and β curves for the bacterium *Staphylococcus albus*. The α curve shows considerable curvature before becoming exponential but the β curve is exponential along its entire length. The cell cycle times of sister pairs were then ranked by Ti (a procedure suggested by R. Peto) the cycle time of the youngest of a sibling pair and separate β curves constructed for groups of sister cells where 2 min $< Ti <$ 28 min, 29 min $\leq Ti <$ 34 min, 34 min $\leq Ti <$ 39 min and 39 min $\leq Ti$. The resulting β curves for these groupings are also shown in Fig. 1. All these β plots are exponential and show no signs of initial curvature. These curves are also parallel to the β plot for the whole population and the exponential tail of the α curve. Similar data have been obtained for SV3T3 cells (not shown) but the data for *S. albus* are used here as they contain a larger number of cell cycle times. If β curves are constructed for pairs of cells chosen at random the β curves are no longer parallel to the α curve and exhibit initial curvature which is especially marked if β curves are constructed for sets of sister cells ranked as described above (not shown). The plotting of β curves for sister cells grouped in this way is a powerful method for determining whether the β curves are exponential or not.

The results presented here and elsewhere[5-8] show that exponential β curves are obtained in practice. Are exponential β curves obtainable in ways other than postulating that the cell cycle contains an exponentially distributed component? That is, what forms of $Q(t)$ in equation (3) can satisfy

$$\beta(T) = \exp(-kT) = -2\int_{t_1}^{t_2} Q(t+T)\dot{Q}(t)\,dt \quad (5)$$

Differentiating (5) with respect to T

$$-k(\exp(-kT)) = -2\int_{t_1}^{t_2} \dot{Q}(t+T)\dot{Q}(t)\,dt \quad (6)$$

substituting for e^{-kt} from equation (5) and rearranging we get

$$\int_{t_1}^{t_2} \dot{Q}(t)\{\dot{Q}(t+T) + kQ(t+T)\}\,dt = 0 \quad (7)$$

The experiment shown in Fig. 1 shows that exponential β curves are obtained for all sister cells irrespective of the cell-cycle time of the youngest of the cell pair. The limits of integration of equation (5) are thus arbitrary once $t_1 > t_{min}$ the minimum cycle time. The only nontrivial solution of equation (7) is that $Q(t) = \exp(-kt)$. In other words, exponential β curves can only arise if there is an exponentially distributed phase in the cell cycle.

It could be argued that other models of the cell cycle may produce β curves that while not true exponentials are so close to exponential that it would be impossible to tell the difference. Cell cycle models have been proposed that give cycle time distributions that are log normal or reciprocal normal[4]. To test whether these distributions could give exponential looking β curves sets of both log normal and reciprocal normal distributions were generated. The values of cell-cycle times chosen were close to those observed in practice with 3T6 cells growing at different rates[6]. The simulated α curves are shown in Fig. 2 together with β curves calculated by randomly associating 'cells' from the α curve. The α curves obtained in this way are quite close to the types of α curves observed in practice; however the β curves are different. Whilst these are often quite closely parallel to the α curves they all exhibit some initial curvature. This curvature is especially apparent when the β curves are calculated separately for cells ranked according to the youngest cell of the cell pair (inset to Fig. 2).

In the simulation shown in Fig. 2 the β curves were generated by choosing 'sister' cells at random from log normal or reciprocal normal distributions. These simulations are subject to two sources of bias. (1) The form of the distribution of cycle times was assumed whereas in fact it is not known. These particular distributions were chosen as they are currently the

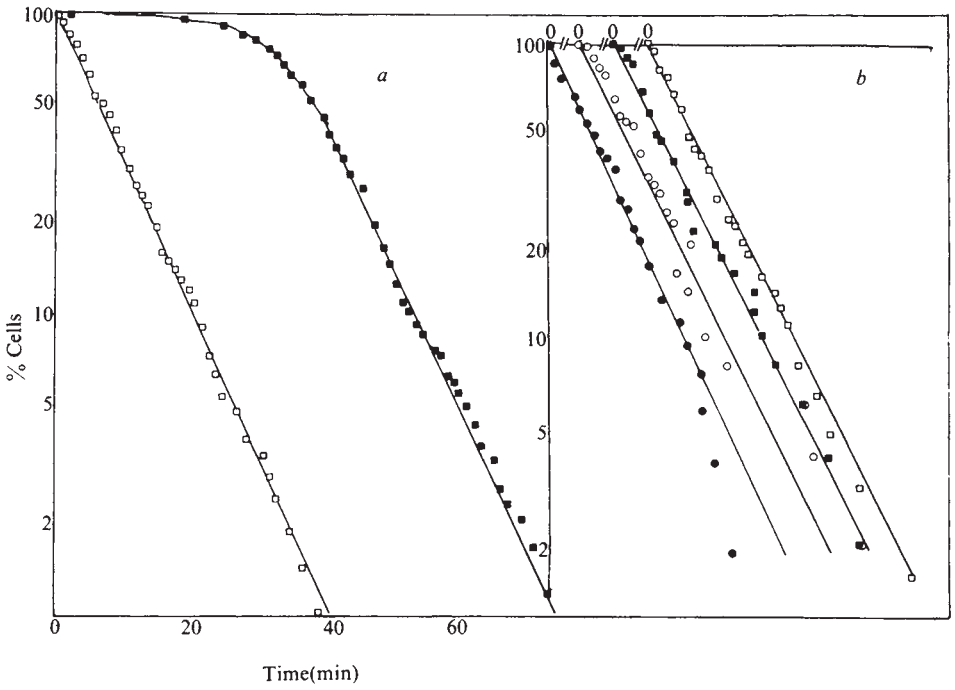

Fig. 1 *a*, Semilogarithmic plots of the % of cells (α_t) undivided against age (t) for *S. albus* (■), together with the corresponding plot of the % of sibling pairs (β_t) with a difference in intermitotic times $\geq t$. The data were obtained from geneologies of *S. albus*. provided by E. O. Powell. There are 439 observations of cycle time in the α curve and 210 intersibling times in the β curve. *b*, β Curves obtained from groups of sister cells ranked according to Ti—the age at division of the earliest dividing cell in the cell pair. ●, 2 min $< Ti <$ 28 min; ○, 29 min $\leq Ti <$ 34 min; ■, 34 $\leq Ti <$ 39 min and □, 39 min $< Ti$. There were 50 cell pairs in each group except (□) which contained 60 cells.

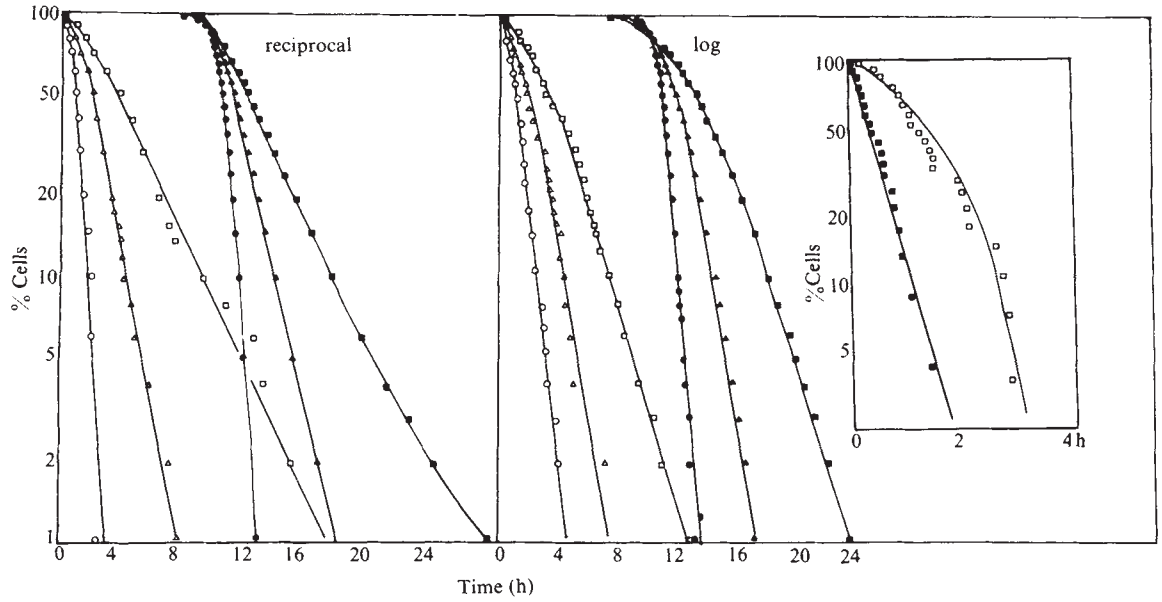

Fig. 2 Calculated α and β curves for reciprocal normal distribution of cell cycle times (left) and logarithmically normal distributions (right). The distributions were generated by transformation of 100 normally distributed numbers with zero mean and unit variance. This procedure was used to generate 100 reciprocally normal or logarithmically normal distributed numbers with chosen means and variance (closed symbols). These 100 artificial cell cycle times were tabulated and associated at random with the aid of lists of pairs of random numbers between 1 and 100 to give 'sister pairs' and the β curves calculated (corresponding open symbols). The inset shows the β curves obtained by ranking the 'cell' pairs by the 'cycle time' of the youngest sister. Fifty 'cell pairs' were created by randomly associating the 100 cycle times shown by the closed circles of the log normal distribution. These were ranked according to the age of the youngest cell of the cell pair. □, The earliest 25 pairs; ■, the last 25 pairs.

most favoured[1,2]. And (2), sister cells were chosen from the distribution at random, that is, their cycle times were uncorrelated whereas in practice sister cell cycle times are frequently correlated. Can more exponential looking β curves be generated if these biases are removed? The bias due to the unknown form of the distribution of cycle times can be overcome by using experimentally measured cycle times, and the bias due to the sister–sister correlations by introducing this correlation into the real data. Cell cycle times were obtained using published geneologies for mouse L cells (ref. 9, Fig. 5) and α and β curves calculated (Fig. 3*a*). 'Sister' cells were chosen at random or by generating correlated 'sister' cells from the α distribution; β curves for random and correlated cells together with the experimentally observed β curve are shown in Fig. 3*b*. The β curve using randomly chosen cells shows initial curvature and then becomes parallel to the observed β curve, the β curve for correlated 'sister' cells shows less curvature but is clearly steeper than the real β curve. While it may be possible in some instances to select correlated cells from an α distribution to give a reasonable facsimile of an exponential β curve this result shows that this is not a general mechanism for doing this. The results presented here and elsewhere[5-8] suggest that the correlation between sister cells arises because the cell cycle is composed of two parts, an identical B phase (respon-

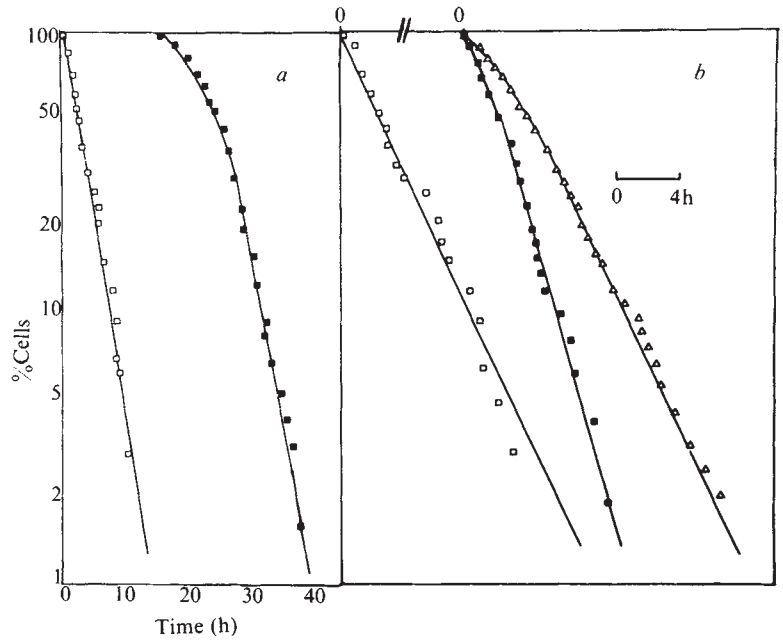

Fig. 3 *a*, α(■) and β (□) plots for published geneologies of L cells (ref. 9, Fig. 5). Synthetic cell-cycle times of 'sister' pairs were generated by multiplying each real cell cycle time (t_r) by $(1+n_r)$ and $(1-n_r)$ where each n_r is a number drawn at random from a normally distributed set of numbers with zero mean and given standard deviation. These numbers were generated using a program designed to generate normally distributed random numbers on an HP97 desk top calculator. The value of the standard deviation used was 0.065 which was determined by trial and error to lead to 'sister' cell cycle times with a correlation coefficient of 0.77 (actual value 0.78). The α curve for these 'sisters' is indistinguishable from the actual α curve (not shown). *b*: □, The experimentally observed β curve; △, the β curve generated from randomly associating real cell cycle times; ■, the β curve generated by introducing the sister–sister correlation. (Note the change of scale.)

sible for the sister–sister correlation) and an exponentially distributed A phase. The β curves then represent the distribution of A phase deviations within the population.

The results presented here show that exponential β curves are a unique property of cell-cycle models containing an exponentially distributed phase. Exponential β curves cannot be obtained from log normal or reciprocal normal distributions of cell cycle duration, nor can they be obtained in general by selecting correlated cells from the actual cell cycle distribution. The most physiologically plausible explanation for the exponentially distributed phase is that the cell cycle contains a random transition.

I thank Dr E. O. Powell for allowing access to his laboratory notebooks and Richard Peto for helpful discussions.

ROBERT SHIELDS

Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London WC2, UK

Received 3 January; accepted 27 April 1978.

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Activated macrophages kill tumour cells by releasing arginase

WHEN rodent peritoneal exudate macrophages are exposed to stimuli such as zymosan, bacterial lipopolysaccharide (LPS) or lymphokines they become cytotoxic to a variety of cultured target cells[1,2]. The toxic effects of such 'activated' macrophages show no immunological specificity but may show a broad selectivity for transformed[3] or malignant cells[4]. Furthermore, this cytotoxic activity has been attributed[4] to a soluble macrophage product. Kung *et al.* have shown[5] that the addition of excess macrophages to mouse mixed leukocyte cultures depletes the culture medium of arginine and thereby suppresses lymphocyte reactivity. This is associated with the appearance of arginase within cultured macrophages. The experiments reported here show that activation of macrophages by zymosan or LPS induces the production and release of arginase and that the cytotoxic activity of the macrophages and of their supernatant media on the target cells is a consequence of arginine deprivation.

Peritoneal exudate cells were obtained by lavage from 10-week-old normal female CBA mice and were seeded at 10^6 per ml into 25-cm^2 plastic culture flasks in Fischer's medium. Following incubation for one hour at 37°C the cultures were extensively and vigorously washed with medium to remove nonadherent cells. The adherent macrophages were then treated with zymosan 50 μg ml^{-1} (Sigma), LPS 25 μg ml^{-1} (from *Escherichia coli* 055:B5, Difco) or control Fischer's medium. Arginase content of the cells was assayed[6] at various times and the results are shown in Table 1. Normal untreated macrophages contained no detectable arginase activity at 0 and 24 h, but after 72 h detectable enzyme activity appeared. Following the addition of LPS there was a prompt appearance of substantial enzyme activity, whereas zymosan treatment induced a relatively modest increase. Arginase activity was readily detected in the supernatant medium from LPS and zymosan treated macrophages but not from the normal macrophages (even after 72 h incubation). Thioglycollate-induced macrophages (collected three days after intraperitoneal injection of 1 ml thioglycollate medium) did not contain or release detectable arginase unless exposed to LPS or incubated for three days, which contrasts with the work of Kung *et al.*[5].

Table 1 Serial assays of arginase content of CBA mouse macrophages following exposure to control medium, zymosan or LPS

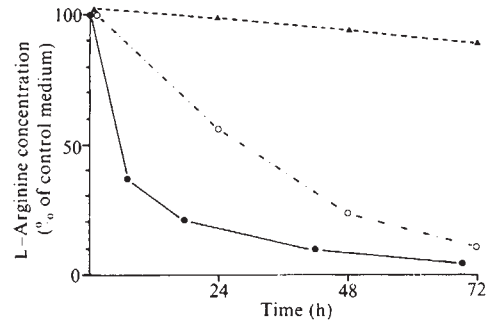
Stimulus	Time (h)	Arginase (nmol urea per min per 10^6 cells)
Nil	0	0
	24	0
	72	34.13
LPS	24	35.4
	72	4.26
Zymosan	24	7.7
	72	8.5

Cultures of peritoneal exudate macrophages (approximately 5×10^6 per flask) were collected by extensive washing in saline and lysed by exposure to distilled water at room temperature for 20 min (plus 1,000 units ml^{-1} Trasylol). The lysates were then centrifuged and the supernatants assayed for arginase content following manganese activation as described by Herzfeld and Raper[6].

The effect of unstimulated and LPS or zymosan-treated macrophages on the amino acid content of the supernatant media was examined. There was a rapid and extensive fall in arginine levels in the supernatant medium from zymosan and LPS treated macrophages, whereas normal macrophages had no significant effect (Fig. 1). Apart from a modest rise in lysine and glutamate levels and a similarly modest fall in serine there was no major effect on the other 11 amino acids measured.

The LPS or zymosan treated macrophages were highly cytotoxic for V79 Chinese hamster lung cells in a colony inhibition assay, as were 24- or 48-h supernatant media from the macrophages. Normal unstimulated macrophages or their supernatant media had no cytotoxic activity, and thioglycollate-induced macrophages were similarly inactive. Supernatant medium (Fischer's medium plus 10% *Mycoplasma*-free fetal calf serum, which contained no detectable arginase) from zymosan treated macrophages incubated for 48 h was shown to be cytotoxic for V79 cells and to contain arginase activity. This medium was then fractionated on a Sephadex G-200 column and the fractions assayed for arginase activity and for cytotoxic effects on V79 cells. Figure 2 shows that the peaks of both activities were coincident, and from previous calibration the molecular weight of the peak was around 120,000 (assuming the activity to be protein). Arginases derived from such sources as rat liver are known to be of similar size[7]. Bovine liver arginase (Sigma), when added to cultures of V79 cells at concentrations similar to those found in macrophage super-

Fig. 1 Sequential levels of arginine in macrophage supernatant media (RPMI 1640). The results are expressed as % of control medium and show a rapid and pronounced fall in arginine concentration in the supernatants from LPS (●) and zymosan (○) treated CBA mouse macrophages, whereas the concentration in the supernatant from unstimulated macrophages (▲) showed no such fall.



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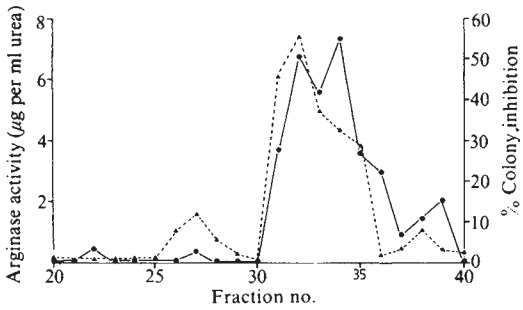

Fig. 2 Fractions 20 to 40 from a G-200 Sephadex column chromatograph of zymosan-treated mouse macrophage 48-h supernatant medium showing arginase activity (▲) and colony inhibition (●) of V79 cells. 48 h supernatants from zymosan-treated CBA mouse macrophages were pooled (20 ml) and then concentrated on an Amicon PM 10 membrane filter before applying to the G-200 Sephadex column, equilibrated with HEPES (20 mM) buffered saline. 5.5-ml fractions were collected and assayed for arginase activity following manganese activation as described[6]. The same fractions were tested for colony inhibition of V79 cells after dilution 1:5 with Fischer's medium plus 10% fetal bovine serum. The peaks of arginase and colony inhibition activity are coincident and were located at an elution volume corresponding to a molecular weight of 120,000 (from previous calibrations).

natants, was highly cytotoxic and this activity was abrogated by the addition of exogenous L-arginine. Furthermore, the cytotoxic activity of LPS and zymosan activated macrophages and their supernatant media could be inhibited by the addition of L-arginine. However, this inhibition was only detectable when the L-arginine was added within the first 18 h of culture. Furthermore, the addition of L-arginine to intact macrophage cultures required 1,000 $\mu g\ ml^{-1}$ to reduce their colony inhibitory activity to 50% (the inhibition was never complete even at higher concentrations), whereas the activity of supernatants from LPS or zymosan treated macrophages could be completely abrogated by the addition of as little as 100 $\mu g\ ml^{-1}$ L-arginine. Fischer's medium is low in arginine (15 $\mu g\ ml^{-1}$). When cytotoxicity experiments were carried out in RPMI 1640 (containing 200 $\mu g\ ml^{-1}$ L-arginine) the macrophage supernatant media were much less active (30% colony inhibition at 1:4) than those produced and tested in Fischer's medium (100% colony inhibition at 1:4).

Table 2 Release of ^{125}I from ^{125}I-iododeoxyuridine-labelled target cells

Source of macrophages	Target cells	Arginine added ($\mu g\ ml^{-1}$) 0	500
Normal female CBA mice	SL_2, DBA_2 mouse lymphoma	49	2
Normal female CBA mice	L5178Y, DBA_2 mouse lymphoma	76	6
Normal female CBA mice	V79, tissue culture line of Chinese hamster lung cells	68	0
Normal female August rat	$ASBP_1$—August rat benzpyrene induced sarcoma	21	0
Normal female August rat	$Xiph_1$—normal adult August xiphisternum mesenchymal cells	0	0

Target cells were exposed to supernatant medium (tested at 1:4) from LPS-treated peritoneal exudate macrophages. The assay was carried out in Fischer's medium plus 10% fetal calf serum with and without additional L-arginine (500 $\mu g\ ml^{-1}$). The lysis assay was carried out as previously described[4] except that the assay was terminated at 18 h. The results are expressed as % ^{125}I release.

To determine whether the V79 cell is unusually susceptible to this form of cytotoxic damage, the effects of activated CBA mouse macrophage supernatants were assessed on other cell types. Such supernatants inhibited the growth and induced lysis of SL_2 and L5178Y lymphoma cells and HSN hooded rat sarcoma cells. LPS-treated August rat macrophage supernatants which contained detectable arginase were lytic to the $ASBP_1$ sarcoma (a benzpyrene-induced August rat fibrosarcoma) as previously described[4], but not to normal August rat fibroblasts, and the tumour-cell lysis was prevented by the addition of L-arginine (see Table 2). All animals and cell lines used in this study were routinely screened for *Mycoplasma* and were negative.

The cytotoxic activity of mouse macrophage supernatant media on V79 cells could be prevented by the addition of L-arginine or L-citrulline, whereas the addition of L-ornithine or putrescine had no effect (data not shown). Arginine is important for at least two distinct key biochemical processes in the cell, one of which is that it is an important precursor of ornithine and the polyamine series. However, neither exogenous L-ornithine nor putrescine could prevent the cytotoxic activity, implying that interference in this pathway is an unlikely mechanism underlying the effects of arginine deprivation. Furthermore, the fact that citrulline could abrogate cytotoxicity suggests that arginine is necessary for protein synthetic pathways essential for cell survival, as citrulline precedes arginine in the urea cycle and can replace arginine in tissue culture media[8].

The release of arginase by activated macrophages may account for many *in vitro* phenomena, such as their effects as suppressor cells on lymphocyte proliferation[9] as well as their capacity to kill tumour cells. The detection of such phenomena will depend on the culture conditions used. Thus, the reported selectivity of macrophage cytotoxic effects[3,4] may reflect differences in the arginine requirements of target cells. The role of arginase release by macrophages *in vivo* is unclear. However, the presence of free arginine would not rule out a local cytotoxic role, for example, within a tumour.

Studies in this laboratory are suported by a programme grant from the MRC. I thank the Cancer Research Campaign for financial support, and Isobel MacCallum for technical assistance. Dr John Walker measured the amino acid levels using a Rank Hilger Chromospeck J180 ion-exchange chromatograph.

G. A. CURRIE

Department of Tumour Immunology,
Chester Beatty Research Institute,
Downs Road,
Belmont, Sutton, Surrey, UK

Received 30 January; accepted 28 April 1978.

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Enhanced NK cell activity in mice injected with interferon and interferon inducers

NATURAL KILLER (NK) cells constitute a distinct subgroup of cells within the immune system[1,2]. They are found in the lymphoid organs of several species including man and are cytolytic on contact in short-term *in vitro* assays for several cell types, in particular tumour cells[1–5]. Although the level of NK cell activity is under genetic control[6,7], several extraneous agents, including bacterial adjuvants, animal viruses and NK-sensitive tumour cells induce an increase in *in vivo* NK cell activity[6–10]. Several of these agents are also known to increase

0028–0836/78/0273—0759$01.00

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Table 1 Effect of interferon inducers on NK cell activity

Cells	100:1*	50:1	25:1
Exp. *a*			
CBA/H spleen			
Control	52.6±1.1§	40.0±0.8	26.0±0.5
Tilorone	92.9±1.8	83.4±1.6	64.1±2.5
Statolon	94.4±1.8	87.3±1.6	67.4±1.2
Exp *b*			
CBA/H spleen			
Control	36.8±2.0	23.2±1.2	12.9±0.5
NDV	72.8±1.4	61.6±0.8	52.0±1.0
Exp. *c*			
C57B16 nu^+/nu^+ spleen			
Control†	45.4±1.8	30.0±1.8	17.1±0.5
Tilorone†	67.0±1.2	51.7±2.0	38.3±3.0
Exp. *d*			
Ig anti-Ig column			
Non-passed cells	61.1	51.3	34.9±
Passed cells‡	74.9	65.7	51.5

Control mice received 0.9% NaCl intravenously or phosphate-buffered saline (PBS)-D orally; four mice were tested individually. In tilorone treated mice, 2 mg of tilorone in PBS-D was given orally 24 h before test. Data represent four individual mice. In statolon treated mice 2 mg of statolon in 0.9 NaCl was given intravenously 24 h before test; four mice tested. For Newcastle disease virus (NDV) treatment, 0.1 ml of NDV containing approximately 10^7 egg infectious $dose_{50}$ (EID_{50}) was inoculated intravenously 24 h before testing for NK cell activity; four individual mice. In exp. *d*, 2 mg of tilorone was given to four CBA/H mice 24 h before test. Spleen cells were pooled and lysed with 0.84% NH_4Cl for 5 min at room temperature. Standard error in this experiment did not exceed 2%. Ig anti-Ig column was prepared and run as described by Wigzell[14]. The increase of NK cell activity after this fractionation procedure is documented in ref. 6.

* Spleen effector to target cell ratio.

† Two individual mice tested.

‡ The cell recovery after passage through the column was 28%.

§ Cells were tested in a 4-h ^{51}Cr-release assay with ^{51}Cr labelled YAC-1 as target cells. Data are presented as specific % lysis according to formula and methods described in ref. 6.

Table 2 Levels of spleen NK cell activity after administration of tilorone and statolon

Group	Time after administration: 2 h	1 day	2 days	4 days	8 days
Tilorone	0.9* (<10)	8.0 (560)	7.0 (<10)	2.5 (<10)	1.2 (<1)
Statolon	ND	7.0 (815)	4.3 (<10)	3.2 (<10)	2.0 (<1)

In the respective treatment groups, 2 mg of tilorone was administered orally and 2 mg of statolon intravenously to four CBA/H mice at the indicated time before test. Spleen cells were tested from individual animals. ND, not determined.

* Lytic index. Cells were tested as described in Table 1§. Lytic units were calculated from the linear part of the dose–response curve to represent the number of cells capable of lysing 50% of the target cell population. The lytic index was calculated by dividing the lytic units from the different spleen cell populations with the control population. Figures in parentheses indicate interferon titres (reciprocal) in serum from mice treated at the indicated times. The test is described in ref. 15. Data represent the mean of four animals.

Table 3 Effect of sheep anti-mouse interferon globulin on the induction of splenic NK cell activity

Cells	100:1§	50:1	25:1
Expt *a*			
Spleen cells			
Control	31.9±0.9	19.6±0.6	10.1±0.3
Tilorone	74.1±1.2	60.2±1.6	42.2±1.6
Tilorone+anti-IF	43.8±1.2	27.6±0.6	14.0±0.4
Expt *b*		100:1§	
Spleen cells			
Control	−0.7±0.3; −0.7±0.2‖		
Anti-IF globulin*	−0.7±0.5; −0.4±0.4		
NDV†	20.9±1.3; 19.9±3.3; 31.5±0.7		
NDV+anti-If	−1.2±0.3; −0.3±0.2; −0.5±0.06		
Tilorone‡	32.0±12; 22.0±2.3; 26.4±2.0		
Tilorone+anti-IF	−0.3±0.2; 5.8±1.2; −0.8±0.1		
Expt *c*	50:1	25:1	12:1
Peritoneal exudate cells			
Control	2.3±0.0‖	2.7±0.0	0.0±0.0
C. parvum	77.0±0.7	70.2±0.0	68.7±1.3
C. parvum +anti-IF	57.9±6.0	50.0±5.8	38.3±5.1

In expt *a* control group, spleen cells from CBA/H mice 6–8 weeks of age were used; four mice tested. In the tilorone group, four mice received 2 mg tilorone orally 24 h before test. The tilorone+anti-IF group was treated similarly, but 0.2 ml of anti-interferon globulin (titre 4×10^{-5}) was injected intravenously at the same time. The method of production and assay of this anti-interferon globulin is described elsewhere[16]. In expt *b*, spleen cells from 2 month old BALB/c male mice were used; three mice were tested individually in triplicate. In expt *c*, peritoneal exudate cells were prepared by flushing the peritoneum with medium containing heparin (5 U ml^{-1}). Control data represent pooled peritoneal cells from four mice. Otherwise, four CBA/H mice received 10 μg of heat-killed *C. parvum* alone intraperitoneally before test, or 0.2 ml of sheep anti-mouse interferon globulin intravenously at the same time.

* Sheep anti-mouse interferon globulin 0.1 ml injected at the same time as the administration of tilorone or NDV.

† 0.1 ml of NDV (approximately 10^7 EID_{50}) injected intravenously.

‡ 4.0 mg of tilorone orally.

§ Effecter to target cell ratio.

‖ See Table 1§.

the resistance of mice to transplantable tumours. This effect may be mediated by NK cells, as a positive correlation exists between *in vivo* resistance to syngeneic tumours and the levels of NK cell activity in the individual mice[11,12]. Viruses as well as several immunoadjuvants are also inducers of interferon. Here we present evidence that interferon and interferon inducers markedly enhance NK cell activity in mice, and suggest that interferon may be the mediator by which many different agents increase NK cell activity *in vivo*.

Tilorone, statolon and Newcastle disease virus (NDV), all potent inducers of interferon in mice, induced a marked increase in spleen cell cytotoxicity (Table 1). The data from several experiments summarised below indicate that NK lymphocytes (and not other potential cytolytic cell types) are responsible for this cytolytic activity: (1) removal of adherent cells (that is, macrophages) from spleen cells (from tilorone-injected mice) by passage through nylon fibre columns[13] not only did not abrogate cytotoxicity but increased the level of cytotoxicity of the unretained cells. (For example, at a 50:1 effector: target cell ratio, there was initially 27% specific lysis, whereas the cytotoxicity of cells recovered after passage through a nylon fibre of column was 42% specific lysis.) (2) Interferon inducers caused a comparable increase in spleen cell cytotoxicity in homozygous athymic nude mice to that in heterozygous mice. (3) NK lymphocytes exhibit a spectrum of cytotoxicity when tested on NK-sensitive or resistant tumour target cells. The induced spleen cytolytic cells exhibit the same spectrum of activity on these target cells as do 'normal' NK lymphocytes. (4) After fractionation of spleen cells on an Ig anti-Ig column[14] an increase in NK cell activity is observed[6]. Likewise, induced cytolytic cells show an increased activity after passage through an Ig anti-Ig column (Table 1).

The kinetics of induction of NK cell activity after injection of tilorone or statolon (Table 2) paralleled the known kinetics of interferon induction. Thus, increased NK cell activity was observed 24 h after inoculation (a time of peak serum interferon levels) but not at two hours (before significant interferon production). NK cell activity decreased progressively in the ensuing days.

The use of potent antiserum to mouse interferon has enabled us to determine the role of endogenous interferon in mediating the biological effects of several inducers of interferon type I, such as viruses[16], poly I:poly C (ref. 17), statolon[17] and tilorone[17]. Injection of mice with this anti-interferon globulin markedly inhibited the capacity of tilorone and NDV to increase NK cell activity *in vivo* (Table 3). Anti-interferon globulin also inhibited (although to a lesser degree) the NK cell activity of peritoneal exudate cells taken from mice injected with *Corynebacterium parvum* (Table 3). (It has been shown by two groups of workers[8,18] that these cells are NK lymphocytes and not activated macrophages[19].) Control mice injected with anti-interferon globulin alone and tested two days later showed the normal background levels of NK cell activity (data not shown).

Table 4 Effect of interferon treatment on NK cell activity

Strain	Treatment	100:1§	50:1	25:1
CBA/H				
	Control	36.8±2.0	23.2±1.2	12.9±0.5
	Interferon†	73.8±1.0	54.5±2.5	41.5±2.6
CBA/H				
	Control	35.2±2.0	ND	ND
	Interferon‡	11.7±0.8	ND	ND
AKR/B				
	Control	28.3±1.3	19.8±0.8	13.0±0.6
	Interferon	55.1±1.5	41.7±1.7	26.8±1.4
BALB/c* nu^+/nu^+				
	Control	ND	1.3±0.5	1.3±1.0
	Interferon	ND	29.6±5.0	24.3±1.5

All controls received 0.2 ml PBS-D at the same time as interferon intraperitoneally; four mice tested. ND, not determined.

* Spleen cells from BALB/c mice 5 weeks of age, tested on L1210 cells passaged *in vitro*.

† 0.2 ml of C-243 cell interferon, prepared and assayed as previously described[20], of titre 3.2×10^{-6} interferon reference units per 0.2 ml, was inoculated intraperitoneally at 48, 24 and 4 h before test. Data represent four mice tested individually.

‡ Two mice were inoculated with 8×10^5 interferon reference units 17 h before test.

§ Spleen cell effector to target cell ratio.

‖ See Table 1§.

Final proof that interferon is responsible for the enhancement of NK cell activity stems from the demonstration that injection of potent mouse interferon preparations (as a single or multiple doses) also led to a marked increase of NK cell activity *in vivo* (Table 4). As in the experiments with interferon inducers (exp. *c*, Table 1), exogenous interferon preparations also increased splenic cell cytotoxicity in athymic nude mice (Table 4).

We therefore conclude that interferon as well as several interferon inducers known to exert antitumour effects *in vivo*[21-23] all markedly enhance NK cell activity in mice. This is of particular interest, as it has been suggested that the antitumour effects of interferon are in part host mediated[24]. Our results would also extend the known effects of interferon on the immune system[25-29] to include NK lymphocytes. Our findings may be important in understanding the mode of action of interferon and interferon inducers in increasing host resistance to a variety of pathogens and tumours.

We thank Dr Wolf H. Fridman for helpful discussions, Mrs Chantal Maury and Mrs Marie-Thérèse Bandu for technical assistance, and Dr G. Mayer, Merrell-National Laboratories, and Dr W. J. Kleinschmidt, Lilly Research Laboratories, for tilorone and statolon. This work was aided by grants from INSERM (ATP 76-75/03, 47-77-79/16 and 47-77-79/13), the Swedish Cancer Society and NIH contract NOI-CB-64033.

MAGNUS GIDLUND
ANDERS ÖRN
HANS WIGZELL

Department of Immunology, Uppsala University Biomedical Center, Box 582, S-751 23 Uppsala, Sweden

ANNA SENIK
ION GRESSER

Laboratories of Cellular Immunology, and Viral Oncology, Institut de Recherches Scientifiques sur le Cancer, B.P. 8–94800 Villejuif, France

Received 3 January; accepted 4 April 1978.

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Antisera to frog immunoglobulins cross-react with a periodate-sensitive cell surface determinant

WHILE investigating surface immunoglobulin (Ig) on frog (*Rana catesbeiana*) lymphocytes by immunofluorescence, we observed, unexpectedly, that several rabbit antisera to frog Ig stained frog thymocytes and all blood cells, including lymphocytes, granulocytes and erythrocytes[1]. Absorption with frog erythrocytes abolished the staining of thymocytes and granulocytes, indicating that similar antigenic determinants are present on these cell types. Some peripheral lymphocytes were still stained by the absorbed antiserum, but others were unstained. Evidently, after removal of the cross-reactive antibodies, two lymphocyte populations, possibly homologous to mammalian and avian B and T lymphocytes, can be identified on the basis of surface Ig. Antibodies that react with all blood

0028–0836/78/0273—0761$01.00

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cells were detected in five of eight antisera to frog high molecular weight (HMW) Ig, but not in six antisera to the antigenically distinct low molecular weight (LMW) Ig. (*Rana catesbeiana* have three distinct Ig isotypes: an HMW Ig, which seems similar to mammalian IgM, and two highly cross-reactive LMW Igs, which have an uncertain relationship to mammalian Ig classes[2,3].) The presence of these cross-reactive antibodies in antisera to HMW Ig may lead to the mistaken identification of Ig on cell surfaces. Absorption of such an antiserum with purified frog HMW Ig and with a non-Ig component of frog plasma, but not with frog LMW Ig, also abolished the staining of thymocytes and granulocytes. The presence of similar determinants on the surfaces of several cell types and on at least two plasma components raised the possibility that a carbohydrate structure might be involved. Experiments supporting this possibility are reported here.

A pool of frog HMW plasma proteins, containing both the HMW Ig and the non-Ig cross-reactive component, was subjected either to periodate oxidation (to degrade carbohydrate determinants) or to reduction and alkylation in guanidine (to degrade protein determinants). After periodate treatment, the sample was at least 70-fold less active than an untreated control in absorbing from an antiserum to HMW Ig antibodies that stain neutrophils, indicating extensive destruction of determinants that are recognised by the cross-reactive antibodies (Table 1). Additional tests with the absorbed antiserum indicated that the staining of thymocytes was similar to that of neutrophils. In contrast, there was no change in the ability of the periodate-treated pool to absorb antibodies that agglutinate sheep erythrocytes coated with HMW Ig. Moreover, in double diffusion, the periodate-treated sample was antigenically identical to the control (Fig. 1). (The absence of a spur indicates that antibodies to the cross-reactive determinant do not precipitate with the untreated antigen.)

After reduction and alkylation in guanidine, the pool no longer formed a precipitin line with the antiserum to HMW Ig (Fig. 1), and it was at least 90 times less active in absorbing, from the same antiserum, antibodies that agglutinate erythrocytes coated with HMW Ig (Table 1). However, the reduced preparation was able to absorb antibodies that stain neutrophils, although it was slightly less active than the control (Table 1). After absorption with this material, the antiserum to HMW Ig stained only the Ig-positive lymphocyte population. Digestion of the reduced preparation with Pronase cleaved almost half the peptide bonds of the constituent proteins and converted them into dialysable peptides. The dialysate was still able to absorb antibodies that stain neutrophils (and thymocytes), although a marginally significant decrease in this activity was observed (Table 1).

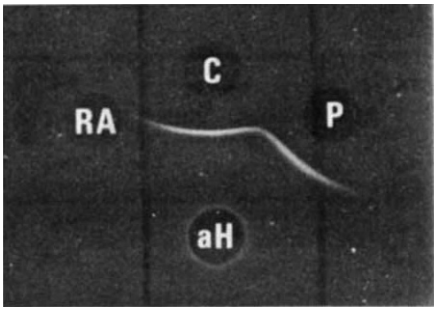

Fig. 1 Effects of periodate oxidation and reduction in guanidine on antigenic reactivity of frog HMW Ig in immunodiffusion. Antigens are at $A_{278} = 2.0$. C, untreated HMW pool from frog plasma. P, periodate-treated[32] HMW pool. The HMW pool ($A_{278} = 11.2$) was dialysed against 0.04 M sodium acetate buffer, *p*H 5.4, 0.1 M NaCl, 0.01 m NaN_3. An equal volume of 0.02 M potassium metaperiodate in the same buffer was added. After 1 h at room temperature, a 20-fold molar excess of glycerol was added, and the sample was dialysed against 70% Dulbecco's balanced salt solution (70% BSS) (Gibco). RA, reduced and alkylated HMW pool. The HMW pool was adjusted to 7.0 M guanidine-HCl, 0.5 M Tris-HCl, *p*H 8.1, 0.1 M dithiothreitol, 1 mM EDTA, and flushed with nitrogen. After 2 h at room temperature, iodoacetamide was added to 0.25 M. After another 2 h at room temperature in the dark, the sample was dialysed against 70% BSS. No significant precipitation occurred during dialysis. aH, one of the antisera to frog HMW Ig that reacts with the cross-reactive determinant.

Table 1 Selective degradation of antigenic determinants in a pool of frog high molecular weight plasma proteins

Treatment	A_{278} units* required to: abolish fluorescent staining of neutrophils	reduce haemagglutinating titre of anti-HMW Ig 100-fold
None	2.4	2.4
Periodate†	>180‡	2.7
Reduction and† alkylation	6.8	>220‡
Pronase digestion (dialysate)	13	>100‡

0.31 ml of serial two-fold dilutions of each preparation was incubated with 0.01 ml of anti-HMW Ig for 3 h at room temperature, then overnight at 4 °C. Any precipitate was removed by centrifugation. A_{278} units required for absorption are expressed relative to 1 ml of undiluted antiserum. The haemagglutination assay with sheep erythrocytes coated with frog HMW Ig and the method for fluorescent staining have been described[1]. The haemagglutinating titre of the unabsorbed antiserum was 2^{15}. For Pronase digestion, 25 μg of enzyme (Calbiochem) was added to 3.0 A_{278} units of reduced, alkylated sample in 0.42 ml 0.2 M Tris-HCl, *p*H 8.0, 10 mM $CaCl_2$, 10 mM NaN_3. After 24 h at 37 °C, the sample was dialysed at 4 °C against an equal volume of distilled water, using #8 dialysis tubing (Union Carbide), and the dialysate was collected. An ^{125}I-mouse IgG marker was added to establish the integrity of the dialysis bag. The material absorbing at 278 nm became entirely dialysable after this digestion. The extent of breakage of peptide bonds was determined by the ninhydrin method[31] to be 46%. A completely hydrolysed control was obtained by incubation in constant boiling HCl in an evacuated tube for 24 h at 110 °C.

* A_{278} of sample in 1 cm path × volume in ml.

† Conditions for periodate oxidation and reduction and alkylation are described in the legend to Fig. 1.

‡ No absorption at the highest concentration tested.

The sensitivity of the cross-reactive determinant to periodate, and its resistance to reduction in guanidine and to Pronase digestion suggest that it may be a carbohydrate. (For simplicity, we refer to a single determinant, although several structures could be involved in the cross-reaction.) It is unlikely that conformational determinants of proteins would survive reduction in a denaturing solvent and protease digestion, although a peptide determinant might retain activity after such treatment. Carbohydrate determinants would probably not be affected. In contrast, periodate oxidation would be expected to modify carbohydrate determinants preferentially, although some alteration of certain amino acid residues may also occur[4–6]. (In our experiments, amino acid analysis of the periodate-treated HMW pool showed 5–10% degradation of half-cystine and tyrosine, but no appreciable change in other residues; tryptophan was not determined.) Conclusive evidence regarding the nature of the cross-reactive determinant will require isolation of the active moiety, and such experiments are planned.

Cross-reactions between oligosaccharide determinants of mammalian IgM and cell surfaces have been suggested in other studies. Merler *et al.*[7] isolated five glycopeptides from human IgM, each containing 5–13 sugar residues and 6–9 amino acids. Antibodies specific for these glycopeptides were prepared by immunoadsorption from a rabbit antiserum to IgM. After conjugation with fluorescein, these antibodies were found to stain the surface of a large fraction of blood or tonsillar lymphocytes. Cells other than lymphocytes were not examined. Ivanyi *et al.*[8]

described a cross-reaction between sheep IgM and erythrocytes that was inhibited by hog blood-group substance, suggesting that the shared determinant is a carbohydrate. This determinant was recognised by antisera to sheep or other mammalian erythrocytes raised in chickens but not in mammals, and was shared or partially shared by erythrocytes and sera of several mammalian species. It has also been observed that in rabbit sera natural antibodies to human tumour cells of several histological types can be absorbed by human IgM (refs. 9, 10).

It seems likely that the earlier observation of fluorescent staining of frog thymocytes by rabbit anti-Ig antibodies[11] could also have been the result of a reaction with the cross-reactive determinant we have described, and does not necessarily mean that Igs are present on the surfaces of these cells. Reports of surface Ig on thymocytes of other poikilotherms[12-16] may have a similar basis. Moreover, the fluorescent staining of mouse thymocytes by chicken antisera to mouse IgM (ref. 17) may also be related to such a cross-reaction. Although rabbit (or other mammalian) antisera to mouse IgM do not usually stain mouse T lymphocytes, low levels of antibodies to a cross-reactive determinant might be present in such antisera and could interfere with T-cell functions such as antigen binding[18]. However, as the cross-reactive determinant is not found on frog LMW Ig, our results are probably not relevant to evidence for light chain determinants on T lymphocytes[18,19].

We speculate that in all vertebrates an oligosaccharide is shared by IgM and a membrane component of thymocytes and blood cells, and perhaps of all cells. A strong antibody response to this oligosaccharide is usually obtained only by immunising phylogenetically distant species. Carbohydrate antigens, however, may be sporadically distributed among species and tissues[20-23], and it is possible that in some species, the postulated immunogenic oligosaccharide of IgM is found only on certain cell types. The presence of the same carbohydrate determinant on different molecules has been shown for human ABH and Lewis blood-group antigens. These oligosaccharides are conjugated either to polypeptides or to lipids and are found both in secretions and on cell surfaces[22,23].

The constant region of the human μ chain contains five oligosaccharides; their location on the polypeptide chain is known[24,25], and they have been partially characterised[26-28]. Results of Hogg and Greaves[29] provide a basis for speculating on the identity of the postulated immunogenic oligosaccharide. They found that certain class-specific antisera to mouse IgM inhibited antigen binding by T lymphocytes. The antibodies responsible for this activity were absorbed by $F(ab')_2$ fragments, but not by Fab fragments of IgM. The $F(ab')_2$ fragment, but not the Fab fragment, would be expected to contain the C2 oligosaccharide[24,25,30].

We thank S. Turco for advice on periodate oxidation. This work was supported by grants from the NIH.

M. JULES MATTES
LISA A. STEINER

Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received 30 January; accepted 17 April 1978.

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V and *C* parts of immunoglobulin κ-chain genes are separate in myeloma

DNA SEGMENTS coding for amino terminal and carboxyl terminal half of immunoglobulin chains are separate in the embryo, and specific rearrangement in these DNA segments occurs during differentiation of lymphocytes[1-3]. Although the simplest model would be that the rearrangement brings a *V* gene in contiguity with a *C* gene, thereby allowing RNA polymerase to transcribe a whole immunoglobulin gene continuously, it has not been directly shown that this is really the case. One way to study this problem and which we report here is to hybridise DNA from a plasmacytoma with a purified light chain mRNA in conditions which would not permit renaturation of the gene segments, digest all single-stranded nucleic acid with single strand specific nuclease S_1 (ref. 4), and determine the size of the DNA segment which was protected by the mRNA from digestion with S_1 nuclease. Using the fact that the length of the immunoglobulin mRNA and its two regions corresponding to *V* and *C* genes are known with considerable accuracy[5,6], we have been able to determine that *V* and *C* genes are not contiguous.

We first confirmed that S_1 nuclease does not degrade DNA–RNA hybrids in our digestion conditions by carrying out the following model experiment. ^{32}P-labelled, complementary DNA was synthesised on a purified κ-chain mRNA (MOPC 321) template and the full transcript was isolated. The DNA was annealed with the excess κ-mRNA and the hybrids, after treatment with the S_1 nuclease, were fractionated by acrylamide gel electrophoresis in 98% formamide. Figure 1 shows that the overwhelming majority of the ^{32}P–cDNA remains intact when the cDNA is preannealed with excess κ-mRNA and subsequently treated with S_1 nuclease.

V and *C* genes can be arranged in three possible ways in the plasmacytoma DNA, depending on whether or not these genes are contiguous with each other and depending on whether or not the 5′and 3′ untranslated sequences are contiguous with *V* and *C* translated sequences, respectively. To distinguish between the three possible arrangements shown in Fig. 2, we did a protection experiment similar to that described above using DNA from plasmacytoma MOPC 321 and purified, homologous κ-mRNA. We digested the MOPC 321 DNA with a restriction enzyme and fractionated the digest on 0.9% agarose slab gels, as previously described[1]. Figure 3 shows the pattern of hybridisation of an *Eco*RI digest of MOPC 321 DNA with homologous, iodinated κ-mRNA. A hybridisation pattern of 18S rRNA is also shown to illustrate complete digestion[9].

Gel fractions with an average molecular weight of 11 megadaltons were pooled as indicated. The pooled DNA should contain the V_κ and C_κ genes, as it hybridised with both whole and 3′ half mRNA, and as other regions on the gel hybridised with either of the two RNA probes only to the extent which can be attributed to 'background' counts that are

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roughly proportional to the optical density profile. The pooled DNA fragments were annealed to an excess of MOPC 321 κ-mRNA. The single-stranded moiety of the hybrid was digested with S_1 nuclease. The nuclease resistant nucleic acids were treated with 0.2 M NaOH at 45°C for 30 min to digest RNA. The remaining DNA was fractionated in acrylamide gel in denaturing conditions. The gel was cut, and DNA from each fraction was hybridised with iodinated MOPC 321 to localise the position of the complementary DNA strand. Figure 4 shows that a DNA fragment of 0.19 megadaltons (590 bases) was protected from S_1 digestion by hybridised mRNA. This is about half of the estimated length of the κ light chain mRNA minus the poly A track, suggesting that the *V* and *C* genes in MOPC 321 plasmacytoma are not contiguous. However, the result could also be obtained if there were an *Eco*RI site near or at the *V–C* joint of a continuous κ-chain gene. This, however, is incompatible with the amino acid sequence[10].

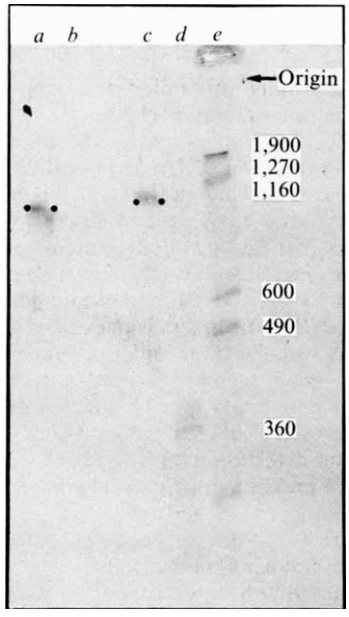


Fig. 1 Protection against S_1 nuclease of cDNA by preannealing with complementary κ-mRNA. ^{32}P-labelled DNA complementary to purified MOPC 321 κ-mRNA was synthesised by reverse transcriptase. The incubation mixture (in 0.2 ml) consisted of: 50 mM Tris-HCl (*p*H 8.3), 10 mM $MgCl_2$, 10 mM dithiothreitol, 4 mM tetra-natrium-pyrophosphate, 1 mM each of dATP, dCTP, dGTP, dTTP, 200 μCi dATP (250 Ci mmol^{-1}), 5 μg mRNA, 2 μg oligo dT_{12-18} (P.-L. Biochemicals) and 10 units AMV reverse transcriptase. Incubation was at 46°C for 10 min. The reaction product was incubated for 12 h in 0.3 M NaOH at 37°C, neutralised, extracted with phenol and passed through a 3-ml Sephadex G-150 column in H_2O. The excluded material (cDNA) was fractionated by electrophoresis in 5% acrylamide gel in 98% formamide[7]. The cDNA in the major band (~1,000 bases long) was extracted and incubated with and without excess purified κ-mRNA (0.2 μg) at 70°C for 3 h. Beside the mRNA, the annealing mixture contained in 20 μl: 225 mM $NaPO_4$, *p*H 7.5, and 3,000 c.p.m. ^{32}P-labelled cDNA (specific activity 10^6 c.p.m. μg^{-1}). After annealing, the sample was diluted 10-fold with the following mixture: 50 mM Na-acetate, *p*H 4.2, 250 mM NaCl, 1 mM $ZnSO_4$, and 20 μg ml^{-1} denatured, sonicated calf thymus DNA. The final *p*H and [Na^+] concentration were 4.6 and 300 mM, respectively. The mixtures were incubated at 45°C for 30 min in the presence of 15 units S_1 nuclease (prepared by the method of Vogt[8]), phenolised, concentrated by alcohol precipitation and electrophoresed in 5% acrylamide gel (1.5 mm thick, 20 cm long) in 98% formamide. The gel was exposed to Kodak X-Omat R film. *Hin*dIII or *Hae*III digested, end-labelled SV40 DNA fragments were electrophoresed in parallel as size markers. Channel *a*, cDNA incubated with mRNA followed by S_1 treatment; channel *b*, cDNA incubated without mRNA followed by S_1 treatment; channel *c*, cDNA without any treatment; channel *d*, *Hae*III-digested SV40; channel *e*, *Hin*dIII-digested SV40.

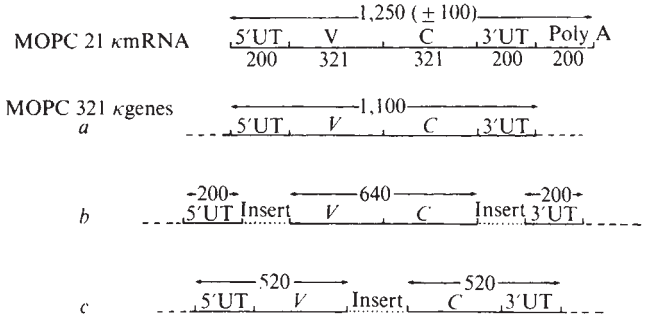
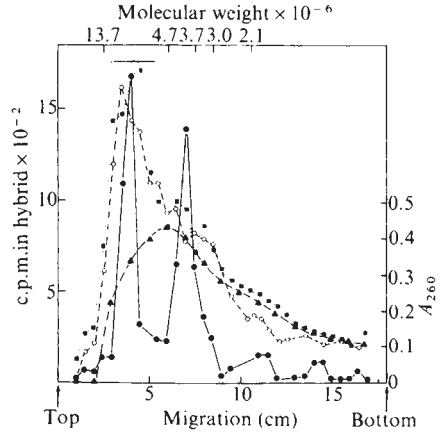


Fig. 2 Different possible arrangements of *V* and *C* translated and 5′ and 3′ untranslated sequences of MOPC 321 κ-chain genes. Length in numbers of bases is indicated for each DNA segment and derived from the known numbers for MOPC 21 mRNA (ref. 6).

Absence of such an *Eco*RI site was confirmed by our recent studies in which double-stranded, full-size DNA which was synthesised on MOPC 321 κ-mRNA template using reverse transcriptase and DNA polymerase, was treated with this restriction enzyme (unpublished observation).

The protection experiment data could be compatible with the arrangement shown in model *b* of Fig. 2. In this case, mRNA would protect a sequence of about 640 bases, which is not very different from what was actually observed. To consider this possibility further, we did the following experiment. MOPC 321 DNA digested with restriction enzyme *Bam*HI was fractionated on 0.9% agarose gel and the 2.4-megadalton component which hybridises to both whole and 3′ half mRNA was isolated[1]. Our more recent studies on a DNA clone which contains the nearly full sequence of the MOPC 321 κ-mRNA demonstrated that *Bam*HI cleaves the *V* gene DNA at positions corresponding to amino acid residues 64 and/or 66, and 99, and that the same enzyme has no cleavage sites in the *C* gene (unpublished observation). The cleavage site corresponding to amino acid residue 99 is very close to the *V–C* junction. Thus, the 2.4-megadalton DNA component should not contain on the same fragment both *V* and *C* gene sequences. On the other hand, as the 3′ end half fragment of the κ-mRNA hybridises with this DNA component[1], it should at least contain the C_κ-gene sequence. Figure 4 shows that a 590-base long sequence of the 2.4-megadalton DNA

Fig. 3 Gel electrophoresis pattern of MOPC 321 DNA generated by *Eco*RI digestion. ^{125}I-labelled whole κ-mRNA of MOPC 321 (○) and 3′ end half fragment[12] (■) (5×10^7 c.p.m. μg^{-1}) were annealed with DNA extracted from gel slices as described[1]. The profile obtained with ^{125}I 18S rRNA is shown for comparison (●). The molecular weight scale was obtained from phage λ DNA, digested by *Eco*RI, which was electrophoresed in parallel. The 11-megadalton fraction of DNA used in the protection experiment is indicated by the bracket. ▲, optical density at 260 Å.



component was protected from S_1 digestion by MOPC 321 κ-mRNA. The length of the protected DNA sequence is too large to be accounted for by model *b*. On the other hand, it agrees well with the notion that the translated and untranslated parts of the C_κ gene are contiguous. In addition, the fact that the hybridisation extent with this protected DNA is about half that obtained with the *Eco*RI-digested DNA is consistent with model *c*.

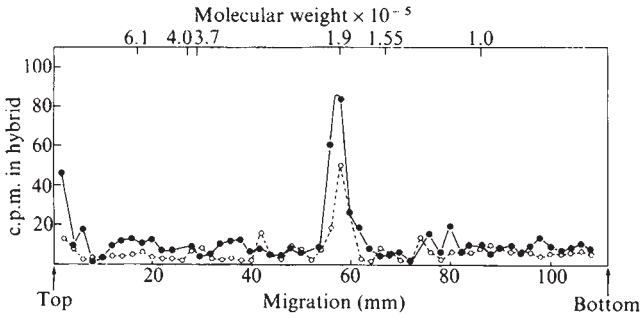


Fig. 4 MOPC 321 DNA (2 mg) digested with *Eco*RI was fractionated on 0.9% agarose as described in Fig. 3. The 11-megadalton fraction (140 μg) recovered from 2 mg total DNA was denatured in 0.1 M NaOH at 37°C for 15 min, neutralised and incubated with an excess of MOPC 321 κ-mRNA (1 μg in 0.2 ml final volume) in 6×SSC/50% formamide at 45°C for 45 min. The control experiment consisted of the 11-megadalton MOPC 321 DNA fraction (160 μg) also recovered from 2 mg total DNA digested with *Eco*RI, and incubated in the absence of mRNA. After treating with an excess of S_1 for 30 min at 45°C (as described in the legend of Fig. 1) and digesting the RNA in the hybrid by alkaline treatment, the S_1 resistant DNA was fractionated on a 5% acrylamide gel in 98% formamide. The gel was cut into 2-mm slices, DNA was eluted by homogenising, freezing and thawing, and each fraction was hybridised with 800 c.p.m. ^{125}I κ-mRNA (7.5×10^7 c.p.m. μg^{-1}) in a 20-μl mixture consisting of 2×SSC, 20 mM Tris-HCl, *p*H 7, 2 mM EDTA at 70°C for 44 h. Data are expressed as a difference in counts between the experimental sample and the control sample (●). An analogous experiment was done using the 2.4-megadalton DNA fragment obtained by digesting MOPC 321 DNA with *Bam*HI (ref. 1) (○). The molecular weight scale was obtained from SV40 DNA digested by *Hin*dIII.

Our results indicate that the κ-mRNA from MOPC 321 myeloma contains stretches of polynucleotides which are transcribed from two DNA segments of about equal length that lie separate in the genome. These segments correspond largely to *V* and *C* gene sequences. We recently reached an analogous conclusion for a λ-chain mRNA, based on evidence obtained by a more direct procedure, namely, by analysis of the DNA fragment cloned from the myeloma cells[11]. In addition, our more recent studies on the MOPC 321 κ-gene clones directly confirmed that the V_κ and the C_κ sequences are separate in the myeloma genome (R. Lenhard-Schuller, C. Brack, B. Hohn and S.T., in preparation). The split nature of coding sequences is not unique to immunoglobulin genes; there are many reported examples[13–18].

After completion of the present studies, we learned that another group had reached the same conclusion based on experiments that are very similar to those described here[19].

We thank Dr J. W. Beard for AMV reverse transcriptase and acknowledge the technical assistance of Ms B. Durrer.

GASTON MATTHYSSENS*
SUSUMU TONEGAWA

Basel Institute for Immunology,
Postfach, CH-4005 Basel 5, Switzerland

Received 28 November 1977; accepted 15 May 1978.

* Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK.

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Generation of prostacyclin by lungs *in vivo* and its release into the arterial circulation

VASCULAR walls generate from prostaglandin endoperoxides or arachidonic acid an anti-aggregatory and a vasodilator substance, prostacyclin (PGI_2)[1,2]. Several prostaglandins are rapidly inactivated as they traverse the pulmonary circulation[3] but prostacyclin passes through unchanged[4,5]. We report here that prostacyclin is continuously generated by the lungs *in vivo* or *in vitro*. Clearly, such an endocrine-like function of the lungs could play an important part in the natural resistance of the organism against intra-arterial thrombosis.

Experiments were carried out in 87 cats anaesthetised with pentobarbitone sodium (40 mg per kg, intraperitoneal); the cats were also heparinised (2,500 IU per kg). A polyethylene cannula was pushed down to the right atrium from the right jugular vein. Through the left carotid artery another cannula was placed in the ascending aorta. Mixed venous blood and aortic blood were withdrawn by a peristaltic pump (3 ml min^{-1}) and, after passage (45 s) through a warmed jacket (37°C), each stream of blood superfused a separate collagen strip which was excised from the Achilles tendon of a rabbit[6]. The blood was

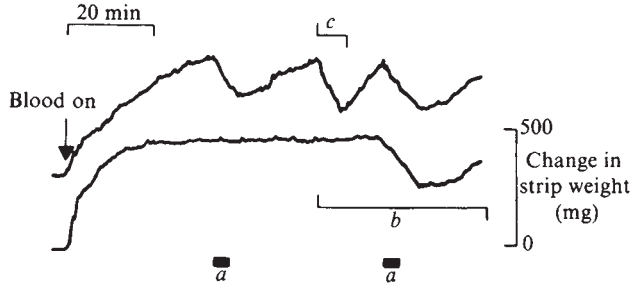

Fig. 1 Quantitation of the release of a prostacyclin (PGI_2)-like substance from the cat lungs. One collagen strip was superfused with mixed venous blood (lower trace) and another with aortic blood (upper trace) (blood on) of an anaesthetised and heparinised cat. The deposition of platelet clumps resulted in an increase in weight (0–500 mg) of the strips. This process was faster in mixed venous blood than in aortic blood. An intravenous infusion of PGI_2 (1 μg per kg over 3 min) (*a*) caused platelet disaggregation in aortic blood, but not in mixed venous blood. However, the same intravenous dose of PGI_2 was effective on both sides of the lungs when a subthreshold disaggregating concentration of PGI_2 (200 pg ml^{-1}) (*b*) was continuously infused into mixed venous blood. Therefore, it was assumed that the difference in concentration of endogenous PGI_2 between mixed venous blood and aortic blood was approximately 200 pg ml^{-1}. The disaggregating response in aortic blood was calibrated with a direct infusion of PGI_2 at a concentration of 800 pg ml^{-1} (*c*).

0028–0836/78/0273—0765$01.00

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Table 1 Disaggregating activity of prostacyclin

PGI_2 concentration ($ng\ ml^{-1}$)	% Disaggregation (no. of experiments) Mixed venous blood	Aortic blood	P
0.1	0 (4)	0 (4)	
0.2	0 (5)	12±1 (5)	<0.001
0.5	0 (6)	31±3 (6)	<0.001
1.0	14±3 (7)	49±6 (7)	<0.001
5.0	38±5 (5)	72±10 (5)	<0.02

Prostacyclin (PGI_2) was infused directly into the heparinised cat blood which superfused the collagen strips. Infusions of prostacyclin were started at least 40 min after the platelet clumps had been formed. Prostacyclin was mixed with blood in the tubing for 45 s before reaching the strips. Data are mean % (±s.e.m.) of the maximal disaggregation induced by prostacyclin. P is the statistical significance of a difference between disaggregating potencies of prostacyclin on a pair of the strips.

then returned to the cat intravenously. The weight of the strips was continuously recorded. The strips gradually increased in weight by 400–600 mg but, after 40 min of superfusion, no further increase in weight occurred (Fig. 1). This gain in weight was due to the deposition of platelet aggregates on the surface of the collagen strips[6].

Infusions of synthetic prostacyclin[7,8], either directly into superfusing blood or into the femoral vein, resulted in a disaggregation of the preformed platelet clumps and consequently in a loss in weight of the blood-superfused strips. The degree of disaggregation was dose dependent and consistently higher in arterial blood than in mixed venous blood (Tables 1 and 2). This difference in disaggregating potency of prostacyclin was still present when mixed venous blood was artificially oxygenated in a silicone fibre oxygenator which introduced a 15-s delay (three experiments). However, the difference disappeared when the cats were pretreated with aspirin (200 mg per kg) or when arterial blood was passed through a delay coil for 10 min before superfusing the tendon (Table 2).

The results can be explained if the lungs continuously release small amounts of an unstable disaggregating substance, the presence of which in aortic blood enhances the disaggregating action of exogenous prostacyclin; the generation of the disaggregating principle in the lungs is inhibited by a high dose of aspirin. The only known endogenous substance that fits the above description is prostacyclin[1,2,5], which has a half life in blood of about 3 min (ref. 5).

Supporting evidence for the continuous generation of prostacyclin-like substance by the lungs is provided by the observation that in all experiments the onset and development of platelet deposition on the collagen strips was slower in aortic blood than in mixed venous blood (Fig. 1). Four experiments similar to that shown in Fig. 1 indicated that the difference in concentration of endogenous prostacyclin in blood 'before' and 'after' the lungs was of the order of 0.1–0.2 $ng\ ml^{-1}$. However,

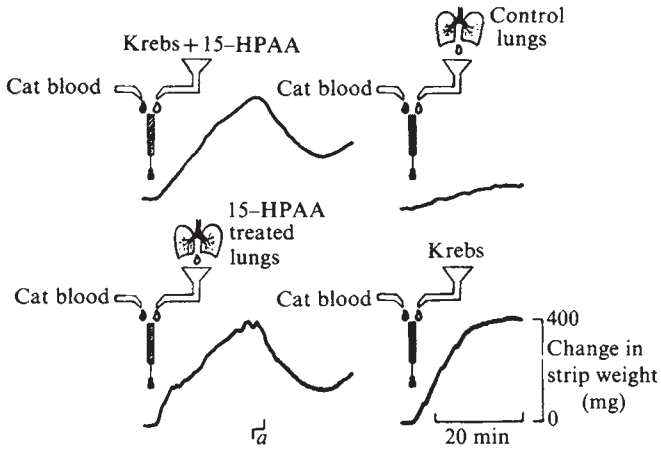

Fig. 2 The generation of an anti-aggregatory principle by isolated perfused lungs of the rat and its blockade by 15-hydroperoxy-arachidonic acid (15-HPAA). One pair of rat isolated lungs (control lungs) was perfused with Krebs' bicarbonate solution (2.3 $ml\ min^{-1}$ at 37°C) and a portion of the perfusate (0.1 $ml\ min^{-1}$) was allowed to drip over a collagen strip which 5 min later was superfused with mixed venous blood of the heparinised cat 3 $ml\ min^{-1}$). Another pair of rat lungs (15-HPAA-treated lungs) was perfused with 15-HPAA (5 $\mu g\ ml^{-1}$) for 30 min before a portion (0.1 $ml\ min^{-1}$) of the perfusate was directed onto the tendon, which 5 min later was treated with cat blood (3 $ml\ min^{-1}$). In the other two experiments, the tendon strips were treated with Krebs' bicarbonate solution (0.1 $ml\ min^{-1}$) or with 15-HPAA (5 $\mu g\ ml^{-1}$) and 5 min later superfused with blood (3 $ml\ min^{-1}$). The pretreatment of the collagen strips with Krebs' bicarbonate solution or with 15-HPAA did not prevent the deposition of the platelet clumps (a gain in weight of up to 400 mg) on the surface of the blood-superfused strips. The formation of platelet aggregates was inhibited by the lung perfusate; however, the release of an anti-aggregatory substance from the perfused lungs was abolished by the pretreatment of the lungs with 15-HPAA, which is a specific inhibitor of prostacyclin synthetase[2]. An infusion of prostacyclin (5 $ng\ ml^{-1}$) (*a*) through the 15-HPAA-treated lungs resulted in the same degree of disaggregation of the preformed platelet clumps as the infusion of prostacyclin directly over the blood-superfused strips. Thus, PGI_2 is not removed in the pulmonary circulation of the 15-HPAA-treated rat lungs.

Table 2 Evidence for the release of prostacyclin by lungs

Dosage and route of administration of prostacyclin	Pretreatment procedure	% Disaggregation ±s.e.m. (no. of experiments) Mixed venous blood	Aortic blood	P
1 μg per kg per 3 min i.v.	None	19±4(9)	47±4(9)	<0.001
5 μg per kg per 3 min i.v.	None	24±4(14)	60±5(3)	<0.05
1 μg per kg per 3 min i.v.	Aspirin 200 mg per kg i.v. 20 min before PGI_2	34±2(7)	38±3(7)	>0.2
5 $ng\ ml^{-1}$ over the blood-superfused platelet clumps	None	38±5(5)	72±10(5)	<0.02
5 $ng\ ml^{-1}$ over the blood-superfused platelet clumps	A 10-min delay in superfusing platelet clumps by aortic blood	41±3(7)	42±4(7)	>0.5

Prostacyclin (PGI_2) was infused into the femoral vein (i.v.) or directly into mixed venous blood or into aortic blood which superfused the preformed platelet clumps. The pretreatment with aspirin equalised the disaggregating potency of PGI_2 (i.v.) in mixed venous blood and in aortic blood. A similar effect was observed when aortic blood was allowed to circulate for 10 min in a warmed (37°C) silicone coil before it reached the strip. Thus, these results suggest that lungs release an unstable de-aggregatory substance, the generation of which is inhibited by aspirin. For other explanations see Table 1.

the direct measurement of the disaggregatory activity of various infusions of prostacyclin (Table 1) suggested even higher concentration differences. Neither type of experiment allowed calculation of the total generation of prostacyclin by the lungs, as prostacyclin is only partially removed from the circulation by the liver and other vascular beds[5].

We have also shown that the isolated perfused lungs of rats and guinea pigs[9] generate spontaneously a substance which, like synthetic prostacyclin, relaxes strips of bovine coronary artery[10] and inhibits platelet aggregation on the collagen strips (Fig. 2). The generation of this substance was inhibited by indomethacin ($10\,\mu g\,ml^{-1}$) and by 15-hydroperoxy-arachidonic acid ($5\mu g\,ml^{-1}$), a specific inhibitor of prostacyclin synthetase[2] (for details see Fig. 2).

Prostaglandins and thromboxane A_2 (TXA_2) are released from the lungs by several nonphysiological stimuli[11]. Our experiments suggest that in the physiological situation the lungs predominantly generate prostacyclin, whereas the generation of TXA_2 is a pathological event, as is the release of histamine[11].

Vane[12,13] has highlighted the metabolic function of the lungs. Substances which are largely inactivated in the pulmonary circulation, including bradykinin, 5-hydroxytryptamine and certain prostaglandins, are unlikely to be circulating hormones[12]. Vane[13] also drew an analogy between the respiratory and metabolic functions of the lungs: "Just as they protect the arterial circulation by removing the waste product, carbon dioxide, so do they remove several vasoactive hormones. Similarly, just as the respiratory function of the lungs adds the vital oxygen to the blood passing through them, so their metabolic activity adds angiotensin II and possibly other vasoactive substances as well". We now postulate that the lungs continuously produce the hormone, prostacyclin. Hormonal prostacyclin circulating in the arterial blood potentiates the anti-platelet and vasodilator activities of the prostacyclin that is generated in the arterial walls[1,2].

Lungs can certainly regulate their production of prostacyclin. We have seen in three experiments that an artificial, vigorous hyperventilation (80 breaths per min) in anaesthetised cats is followed by an increased release from lungs of a disaggregating principle ($0.5–1.0\,ng\,ml^{-1}$ of PGI_2 equivalents). Other physiological stimuli may also exist for the generation of prostacyclin by lungs. On the other hand, tobacco smoking, air pollution and lung diseases could diminish the prostacyclin generating capacity of the lungs and thus disturb homeostasis in the arterial segment of the circulation.

RYSZARD J. GRYGLEWSKI

RYSZARD KORBUT

ANNA OCETKIEWICZ

Department of Pharmacology,
Copernicus Academy of Medicine in Cracow,
31–531 Cracow, 16 Crzegorzecka, Poland

Received 20 February; accepted 3 May 1978.

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Prostacyclin is a circulating hormone

PROSTACYCLIN (PGI_2) is generated by vascular walls from arachidonic acid or prostaglandin endoperoxides[1]. It is the most potent inhibitor of platelet aggregation so far described, and acts by increasing cyclic AMP in the platelets[2,3], presumably through adenyl cyclase stimulation. The enzyme which synthesises prostacyclin is concentrated in the endothelial lining of the vessels[4]; clearly, platelets could be prevented from clumping onto the walls of healthy vessels by the generation of prostacyclin[5]. Unlike prostaglandin E_2 or $F_{2\alpha}$, prostacyclin is not inactivated as it passes through the pulmonary circulation[6,7], and in dog lungs, *in vivo*, prostacyclin is the major metabolite generated during an infusion of arachidonic acid[8]. In the preceding paper, Gryglewski *et al.*[9] conclude that prostacyclin is continuously generated by and released from cat lungs *in vivo*. We report here that we have confirmed this conclusion in a different species (rabbits) by the use of an antiserum which cross-reacts with prostacyclin and neutralises its anti-aggregating activity[10]. This antiserum (PGI_2 antiserum) was raised in rabbits using a stable analogue of prostacyclin, 5,6-dihydro-prostacyclin (PGI_1), as the hapten, conjugated to bovine serum albumin[10].

Rabbits were anaesthetised with pentobarbitone (30 mg per kg, intravenous) and heparin (2,500 IU per kg, intravenous) was injected just before exteriorisation of blood. Arterial or mixed venous blood from the rabbits was withdrawn continuously at a rate of $3\,ml\,min^{-1}$ from a cartoid artery or a jugular vein by a roller pump and used to superfuse a pair of collagen strips excised from the Achilles tendon of a rabbit as described by Gryglewski *et al.*[11]. The tendons were suspended freely from auxotonic[12] transducers, the outputs of which were displayed on a multi-channel pen recorder. Changes in weight due to cell deposition were recorded as deflections of the pens, which had been previously calibrated in milligrams. After superfusing the tendons, the blood was returned intravenously to the animal by gravity.

When the blood superfusion was started the strips accumulated cell aggregates and increased in weight to a constant value over a period of 35–40 min. The increase was 196 ± 19.8 mg ($n = 12$) for tendons superfused with arterial blood. However, the increase observed for tendons superfused with venous blood (276 ± 18.5 mg; $n = 12$) was significantly greater ($P < 0.05$) and faster.

When prostacyclin was infused into the blood superfusing the tendons, there was disaggregation of cells, with a consequent loss of tendon weight. The prostacyclin infusion had a greater effect in arterial than in venous blood. At $1\,ng\,ml^{-1}$, prostacyclin induced 39.1 ± 5.0 mg ($n = 6$) disaggregation in venous blood but 85.8 ± 6.6 mg ($n = 6$) disaggregation in arterial blood. These results confirm those of Gryglewski *et al.* in the cat[9].

Infusion of PGI_2 antiserum (but not of normal serum) at $10–20\,\mu l\,ml^{-1}$ of blood for 15 min into the blood bathing the tendons inhibited the disaggregating effect of prostacyclin ($1\,ng\,ml^{-1}$) by $77.5 \pm 5.5\%$ ($n = 6$) (Fig. 1). There was little or no effect of the PGI_2 antiserum on disaggregation produced by PGD_2 ($100\,ng\,ml^{-1}$). This prostaglandin inhibits platelet aggregation but is also much less affected by the PGI_2 antiserum *in vitro*[10].

When PGI_2 antiserum was infused into arterial or venous blood superfusing tendons which had already achieved a stable increase in weight through cell aggregation, there was an additional increase in weight. This increase was significantly greater for tendons superfused with arterial blood (49 ± 3.8 mg, $n = 5$) than for those in venous blood (18 ± 7.5 mg, $n = 5$). When tendons were superfused with arterial blood obtained from rabbits treated with a high dose of aspirin (200 mg per kg, intravenous, 10 min before) infusion of PGI_2 antiserum did not induce any further increase in tendon weight.

0028–0836/78/0273—0767$01.00

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In four experiments, PGI_2 antiserum was infused into the arterial blood as soon as superfusion of the tendons was started. The increase in weight over 35 min was then much faster than in the control experiments and was similar in magnitude to that seen in venous blood (Fig. 2).

The superfusion of tendons[11] is a most useful extension of the blood-bathed organ technique of J.R.V.[13], for it allows dynamic interactions between platelets and collagen to be studied. Contact of platelets with the tendons during superfusion initiates adhesion and aggregation onto the tendon. The platelet aggregates (and perhaps other blood cells trapped between platelet clumps) induce an increase in tendon weight which stabilises after 35–40 min. Our experiments clearly show that this stabilisation partly depends on the presence of a circulating substance, the effect of which is neutralised by the antiserum, for infusion of the antiserum brought about a further cell deposition onto the tendons. Moreover, the fact that this additional increase in weight was greater in arterial than in venous blood must mean that the concentration of this circulating substance in arterial blood is higher than that in venous blood. Such a conclusion was also suggested by the slower rate of increase of tendon weight in arterial than in venous blood and the lesser overall increase in weight.

The antiserum used reacts with the natural prostaglandins PGE_1 and PGD_2 and synthetic dihydro-prostacyclin (PGI_1), as well as with prostacyclin. However, because prostacyclin is 30–40 times more potent than any of the other known anti-aggregating prostaglandins, the concentration of antiserum used only neutralised the disaggregating activity of prostacyclin[10]. Gryglewski *et al.*[9] showed that the anti-aggregating principle released from the lungs was unstable, thereby eliminating the possibility that it was PGE_1 or PGI_1. In any case, PGI_1 is not made biosynthetically and there is little or no evidence that PGE_1 can be released from lungs. Thus, we must conclude that the anti-aggregating substance which we detected in arterial blood by the use of the antiserum was prostacyclin.

In the preceding paper, Gryglewski *et al.*[9] showed that aspirin, a prostaglandin synthetase inhibitor[14], equalised the rate of increase of tendon weight in venous and arterial blood, an effect which would be expected if aspirin inhibited release of prostacyclin into the blood passing through the lungs. Our results with aspirin support these conclusions, for the PGI_2 antiserum was ineffective in inducing a further increase in tendon weight after aspirin treatment of the rabbit.

Our experiments demonstrate that both arterial and venous blood contain prostacyclin. The higher basal concentrations in arterial blood support the conclusion of Gryglewski *et al.*[9] that the pulmonary circulation releases prostacyclin into the blood passing through and explain why exogenous infusions of prostacyclin had a greater effect in arterial than in venous blood. The most likely source of this continuous prostacyclin release is the pulmonary endothelial cell[15].

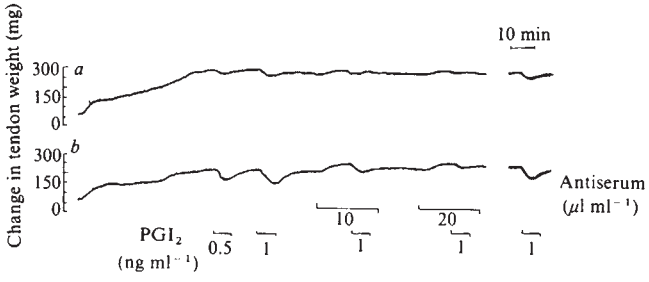

Fig. 1 Effect of PGI_2 antiserum on the disaggregating action of exogenous prostacyclin. One tendon was bathed with venous (*a*) and another with arterial (*b*) blood. After the strips had reached a stable weight, infusion of prostacyclin (0.5 or 1 ng ml^{-1}) induced a dose-dependent reversible disaggregation. There was a greater effect in arterial blood. Infusion of PGI_2 antiserum (10 or 20 μl ml^{-1} blood) induced an increase in weight which was greater for the tendon bathed in arterial blood. The disaggregating effect of prostacyclin (1 ng ml^{-1}) was antagonised. When the infusion of antiserum was stopped, the disaggregating effect of PGI_2 recovered to previous values within 30 min.

Fig. 2 Effect of PGI_2 antiserum on increase in weight of a tendon bathed with arterial blood. The left hand trace shows the increase in weight of a tendon bathed with venous blood, and the right hand traces those of a tendon bathed with arterial blood (*a*) and one bathed with arterial blood containing PGI_2 antiserum at 10 μl ml^{-1} from the beginning of the superfusion (*b*). The weight of this tendon after 35 min of superfusion was greater than the control in arterial blood and similar to the weight of the one bathed with venous blood.

We have previously suggested[5] that prostaclyclin can be formed in blood vessels either from endogenous precursors or from endoperoxides released by platelets attempting to clump onto the vessel wall. It will be difficult to determine which of these mechanisms is of importance in the formation of circulating prostacyclin, for it is well known that platelets, megakaryocytes and other formed elements of the blood can be trapped in the pulmonary circulation.

The presence of circulating prostacyclin provides an explanation for the results of Fleishman *et al.*[16] who observed that aspirin treatment not only inhibited platelet aggregation but slowed disaggregation in a filter loop technique in man.

Thus, our results support the suggestion of Gryglewski *et al.*[9] that the release of prostacyclin from the lungs and its subsequent circulation represents an important homeostatic mechanism for the control of platelet aggregation *in vivo*. These results also confirm the hypothesis of J.R.V. that the lungs can act not only as filters for unwanted vasoactive substances but as generators of circulating hormones[17].

SALVADOR MONCADA
RYSZARD KORBUT
STUART BUNTING
JOHN R. VANE

Department of Prostaglandin Research,
Wellcome Research Laboratories,
Langley Court, Beckenham, Kent, UK

Received 27 February; accepted 3 May 1978.

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Bombesin-like immunoreactivity in the lung

THE first suggestion that the lung contained endocrine cells was made by Feyrter[1] when introducing the concept of a diffuse endocrine or paracrine system, composed of epithelial 'clear cells'. The presence of argyrophil cells in human and other mammalian lungs was later confirmed[2,3] and it was shown that some of these cells contained amines, demonstrated by argent-affinity, formaldehyde-induced fluorescence (FIF), and the possession of neurosecretory type granules. They have variously been termed Feyrter cells[4], Kultschitzky cells[5], argyrophil-fluorescent-granulated or AFG cells[6], enterochromaffin-like cells[7], neurosecretory cells[8], pulmonary argyrophil cells[9], endocrine or endocrine-like cells[10,11], and possess APUD cell characteristics[10-13]. Endocrine cells are present within the bronchial and bronchiolar epithelium both as single elements and as groups of cells. Groups of innervated, endocrine-like cells termed neuroepithelial bodies have been demonstrated[4]. Some pulmonary endocrine cells contain 5-hydroxytryptamine[10,14] and possibly other amines, but no peptide product has yet been identified in them. Said[15] has proposed that the lung may produce a vasoactive peptide and it has subsequently been shown that the lung produces an angiotensin II-like peptide[16,17] and two vasoactive peptides[18], although the cellular origin of these peptides has not been established. Three major types of endocrine cell have been identified in human fetal lung (types 1, 2 and 3), a distinction based largely on the morphology of their secretory granules which are 110–200 nm in diameter[12]. Granules in the type-2 cell of the lung resemble those in P cells of the gut and pancreas[19]. The P-cell product has not been identified but it may be a bombesin-like peptide[20]. Bombesin is a tetradecapeptide isolated from frog skin[21] having a wide range of actions on the mammalian gastrointestinal tract and vasculature[22]. The similarity between P cells of the gut and certain granulated cells of the lung suggested that a bombesin-like peptide might be present in the human lung, and we report here the presence of bombesin-like immunoreactivity in human fetal and neonatal lung.

Human fetal and neonatal lungs were obtained from 25 subjects; three were obtained within 2 h of death, eight within 24 h, seven at 24–30 h, six at 40–56 h and one at 70 h. The subjects included males and females and ranged in age from 8 weeks of gestation to 4 months postnatal. Samples of tissue were taken from adjacent inner, middle and outer regions of the lung for immunocytochemistry (IC) and radioimmunoassay (RIA). For IC, blocks of tissue 3–5 mm thick were quenched in Arcton (Freon) 12, freeze-dried, fixed with benzoquinone vapour[23] and embedded in wax blocks from which 5 μm sections were cut. Tissue for RIA was extracted twice with water and twice with 0.1 M formic acid. Water and acid extracts were separately pooled, lyophilised, reconstituted and assayed at two different dilutions.

Antisera directed to the C-terminal portion of amphibian bombesin and two bombesin analogues were raised in rabbits by immunising them with (1) a carbodiimide coupled amphibian bombesin–bovine serum albumin complex, (2) a glutaraldehyde coupled [Lys^4]bombesin–globulin complex, and (3) a *bis*-diazotised coupled [Tyr^4]bombesin–benzidine complex. The radioactive label was prepared using 0.5 nM ^{125}I, 10 nM [Tyr^9]nonapeptide of amphibian bombesin and 140 nM Chloramine-T, in a potassium phosphate buffer (0.5 M, *p*H 7.4). After 10 s, sodium metabisulphite (200 nM) and potassium iodide (6 μM) were added and the reaction products placed on a Sephadex G-25 column. The column was eluted with 0.1 M formic acid containing 2.0% human serum albumin and 2.5% Trasylol.

The optimal antiserum for RIA was found to be that to pure amphibian bombesin and to the [Lys^4]bombesin analogue, whereas for IC it was antiserum to the [Tyr^4]bombesin analogue.

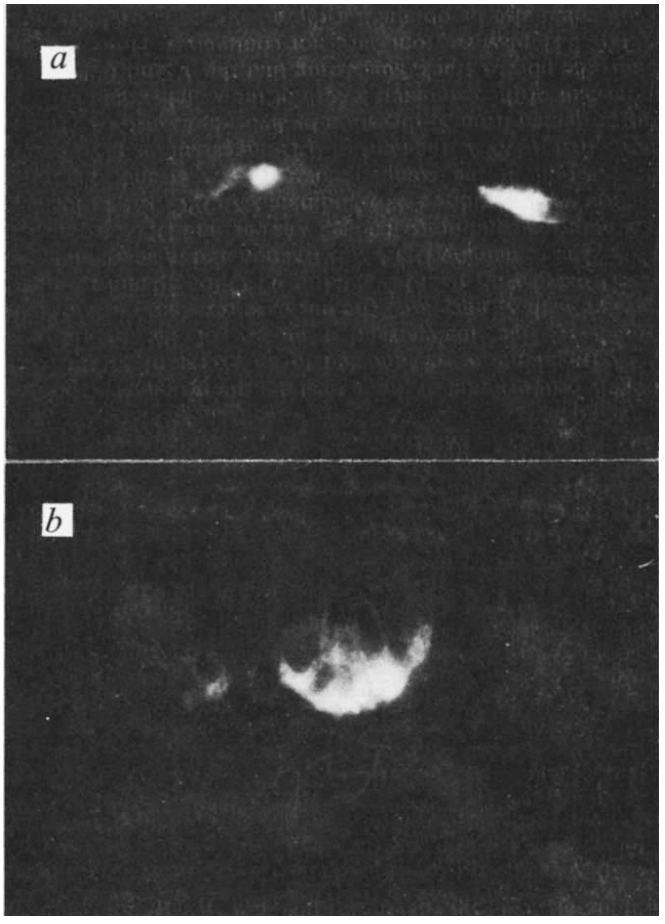
The assay was sensitive to changes of 3 fmol per assay tube and showed no cross reaction with known peptide hormones. The final antibody dilution for the assay was 1/125,000.

For IC, antisera to [Tyr^4]bombesin at a dilution of 1/200, with 24 h incubation, was used. Controls included: (1) the prior absorption of the antibodies with pure bombesin and with other peptides common to brain and gut (see below): (2) application of antibodies to other brain and gut peptides (somatostatin, neurotensin, enkephalin, vasoactive intestinal polypeptide, and substance P): (3) application of normal rabbit serum as first layer and the use of a single layer of fluorescein labelled goat anti-rabbit globulin.

Cells with bombesin-like immunoreactivity were found mainly in the bronchial and bronchiolar epithelium of fetal and neonatal lung, both as single cells and as groups. They were variable in shape and located close to the basement membrane (Fig. 1*a*, *b*). Full quenching of the immunostaining was achieved by the addition of as little as 1.25 nmol ml^{-1} bombesin and not with other neuropeptides. Bombesin-like immunoreactivity was found by RIA to range from 2 to 43 pmol per g wet weight of tissue. In preliminary studies on human adult lung no cells containing bombesin-like immunoreactivity were detected and the concentration of bombesin-like immunoreactivity was found to be <1 pmol per g wet weight of tissue.

The differences between fetal and adult lung in the distribution of bombesin-like immunoreactivity are not surprising as age-dependent changes are known to occur in the endocrine cells of the lung[11]. However, endocrine cells are certainly present in adult lung[5,8,9,11,19,24,25] but they are few in number

Fig. 1 Human fetal lung (25–26 weeks of gestation). Cells with bombesin-like immunoreactivity in bronchiolar epithelium. *a*, Single cells lying along the basement membrane (×1,512). *b*, Group of basally situated cells (×1,512).



0028–0836/78/0273—0769$01.00

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and widely scattered. They seem to be of a single type which may be different from the type-3 cell, which is assumed to be the source of bombesin-like immunoreactivity.

Bombesin has been reported to have a powerful constrictor effect on guinea-pig bronchiolar smooth muscle[26] and bombesin-like immunoreactivity is present in the mammalian gut both in endocrine cells[27] and in nerves[28] and in both the porcine hypothalamus[29] and rat brain[30]. Within the rat brain it is concentrated in the hypothalamus, thalamus and limbic cortex. Bombesin is therefore one of a growing group of peptides common to brain and gut[31]. These neuropeptides have been shown to be present in the periphery within specific endocrine cells. They seem to act both as neurotransmitters and as local or systemic hormones[32,33]. The role of bombesin in the lung can only be guessed at by analogy; it may have a role as a local hormone controlling bronchiolar smooth muscle tone and the local perfusion and ventilation of the lung. The age-dependent distribution of cells containing bombesin-like immunoreactivity suggests that these cells may be of significance during development and birth. Thus, 40 years after Feyrter postulated an endocrine role for the lung, an endocrine peptide has been found in the APUD cells of human foetal and neonatal lung.

This work was supported by grants from the MRC, Cancer Research Campaign, Stiftung Volkswagenwerk and the Council for Tobacco Research. We thank Professor J. A. Will for encouragement and advice. Synthetic bombesin was supplied by Professor R. de Castiglione (Farmitalia).

J. WHARTON
J. M. POLAK*
S. R. BLOOM
M. A. GHATEI
E. SOLCIA†
M. R. BROWN‡
A. G. E. PEARSE

Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12, UK

Received 10 January; accepted 17 April 1978.

* To whom requests should be addressed.
Permanent addresses: † Centro Diagnostica Istopatologica, Universita di Pavia a Varese, Ospedale di Circolo, Viale Borri, 57, 1-21100 Varese, Italy. ‡ The Salk Institute, Laboratories for Neuroendocrinology, PO Box 1809, San Diego, California 92112.

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Biosynthetic relationships of big and little gastrins

ACCUMULATING evidence suggests that many peptide hormones are synthesised initially as part of larger precursor molecules[1]. There is also evidence that several different peptides, for example, corticotrophin, β lipotrophin, the melanotrophins and the endorphins may arise from a single precursor molecule which is processed differently in different secretory systems[2]. The post-translational processing of large precursors would therefore seem to play a regulatory role in determining the biological activity of the final secretory product. We have examined the relationships between the principal biologically active forms of the hormone gastrin in porcine antral mucosa.

These molecules are peptides of 34 amino acid residues (G34, big gastrin) and 17 amino acid residues (G17, little gastrin), both of which exist in sulphated and unsulphated forms[3-5]. They are of interest in the context of post-translational processing because although they are both secreted into blood they differ significantly in their biological properties. In particular, the plasma concentrations of G17 required for a given rate of acid secretion are about five times less than those of G34, and the metabolic clearance rate of G17 is about one-fifth that of G34 (ref. 6). Digestion of G34 with trypsin yields a COOH-terminal fragment identical to G17, and an inactive NH_2-terminal fragment (NT G34) (ref. 5). We show here that antral mucosa contains NT G34 in equimolar amounts with G17 and that both are always present in the same cell, and probably within the same granule. These findings, therefore, directly support the idea that G34 is cleaved intracellularly by a trypsin-like enzyme to produce G17, and draw attention to the importance of this conversion in determining the biological activity of the gastrin released.

It has not previously been possible to identify NT G34 in antral mucosa because it has no biological activity and does not cross-react with antisera raised to G17. However, we immunised rabbits with porcine sulphated G34 conjugated to bovine serum albumin[7] and obtained an antiserum (designated L33) with specificity for the NH_2-terminal part of the molecule which also cross-reacts with NT G34. Initial studies suggested L33 contained both NH_2- and COOH-terminal specific antibodies, and that the latter could be readily removed by

Table 1 Binding of radiolabelled G17 and G34 to antisera*

Antiserum	Label ^{125}I-G34	^{125}I-G17
†L33 (pre-immunoadsorption)	90.1%	66.1%
L33 (post-immunoadsorption)	87.0%	8.6%
1296	82.3%	77.1%
L6	3.8%	82.0%

The antisera were used at the same dilution as those used in immunocytochemistry (see Fig. 2), but similar results were also obtained at the higher dilutions used in radioimmunoasay. For other evidence on specificity see text. Antisera were incubated at 4°C for 48 h in sodium barbitone buffer, *p*H 8.4, 0.025 M, with 2,000 c.p.m. monoiodo ^{125}I-labelled G17 or G34. Antibody-bound label was separated from free peptide by the addition of 20 mg Amberlight IRP 57 resin in the case of ^{125}I-G17, and 20 mg charcoal coated with dextran (10% by weight) and plasma (0.1 ml g^{-1}) in the case of G34.

*Expressed as % of total counts added.

†Immunosorption of L33 was carried out by mixing 1.0 ml antisera for 2 h at 22°C with 1.5 g Sepharose beads to which had been conjugated porcine G17; antiserum was separated from beads by centrifuging. Porcine sulphated G17 (2.0 μmoles) was conjugated to 4 g AH Sepharose beads (Pharmacia) using methods recommended by the manufacturer.

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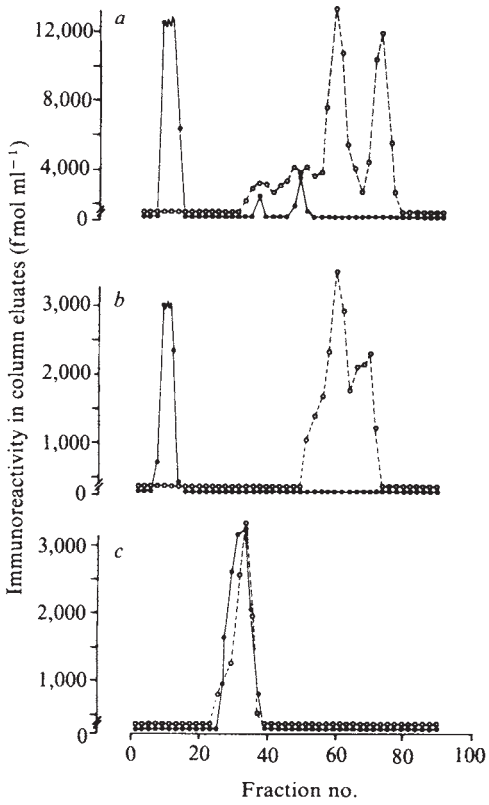

Fig. 1 Separation on AE cellulose columns (1 × 10 cm) of intact unsulphated porcine G34 (*c*), G34 after digestion with tosylphenylethyl-chloromethyl-ketone-treated trypsin (10 μg, 10 min, 20°C) and boiling to inactivate enzymes (*b*), and aqueous extract of hog antral mucosa prepared by boiling tissue in water (0.1 g ml^{-1}, 2 min), homogenising and centrifuging (2000*g*, 10 min) (*a*). Re-extraction of the pellet of tissue extracts indicated that over 90% of immunoreactivity with both 1296 and L33 was recovered in the initial extract. Columns were equilibrated with 0.05 M ammonium bicarbonate and eluted with a gradient to 0.5 M ammonium bicarbonate using a 150-ml conical flask as the mixing vessel. The flow rate was 6 ml h^{-1}, and fractions of 1.0 ml were collected. The column eluates were assayed with L33 (1 : 2,000, treated with G17–Sepharose beads) (●), and with 1296 (1 : 300,000) (○). Previous calibration of these columns indicated that the sulphated form of G34 emerges just after the unsulphated form in tubes 40–50, and that the sulphated form of G17 emerges in tubes 70–80 which is just after unsulphated G17.

immunoadsorption to G17 conjugated to Sepharose beads. Thus, before treatment with the immunoadsorbant, L33 bound both ^{125}I-labelled G34 and ^{125}I-G17, whereas after treatment, binding of ^{125}I-G17, but not ^{125}I-G34, was virtually abolished, indicating removal of COOH-terminal-reacting antibodies (Table 1). Moreover, pure natural porcine G34 (either intact or trypsinised) inhibited binding of ^{125}I-G34 to L33, but G17 had no such effect even at 1,000-fold higher concentrations. Separation of the products of tryptic digestion of G34 by ion exchange chromatography on amino ethyl (AE) cellulose showed the component cross-reacting with L33 to be poorly retained by the column and therefore compatible with the weakly charged NH_2-terminal tryptic peptide (Fig. 1*b*). Radioimmunoassays with a second antiserum (1296) known to be specific for the COOH-terminus of G34 (ref. 8) were used to identify the COOH-terminal tryptic peptide which emerged in a position compatible with G17. As expected, 1296 did not reveal NT G34.

Graded dilutions of boiling water extracts of hog antral mucosa inhibited binding of ^{125}I-G34 to L33 in parallel with standard NT G34. Moreover, when these extracts were fractionated on AE cellulose about 95% of the immunoreactivity with L33 emerged in a single peak in the same position as standard NT G34 (Fig. 1*a*). In contrast, there were two main components detected by 1296 which emerged in positions compatible with the unsulphated and sulphated forms of G17 (Fig. 1*a*). Both L33 and 1296 revealed minor peaks of immunoreactivity emerging between NT G34 and G17 which on the basis of charge and immunochemical properties were identified as the unsulphated and sulphated forms of G34. Using the tryptic peptides of porcine G34 as standards we estimate the concentration of NT G34 in hog antral mucosa to be 7.0 ± 0.4 nmol g^{-1} (mean ±s.e.m., $n = 6$), and that of G17 to be 9.35 ± 0.44 nmol g^{-1}. The presence of nearly equimolar amounts of G17 and NT G34 is consistent with their production by intracellular cleavage of G34, and suggests they should also be sequestered in the same antral cells.

The cellular origins of the gastrins were studied by the indirect immunofluorescent technique of Coons *et al.*[9]. Consecutive serial sections of hog antral mucosa were examined with L33, 1296 and a third antiserum (L6) previously shown to be highly specific for G17 (ref. 10). A similar distribution of gastrin (G-) cells was revealed by all three antisera, and in a count of 304 cells in adjacent serial sections no cells were found that did not stain with each of the antisera. Moreover, at high power the different antisera also revealed similar distributions of secretory granules in the same cell (Fig. 2). The specificity of the immunofluorescence was established by the failure of nonimmune rabbit serum to reveal G-cells, and by inhibition of fluorescence after preincubation of the antisera with appropriate peptides (legend to Fig. 2). In addition, it is

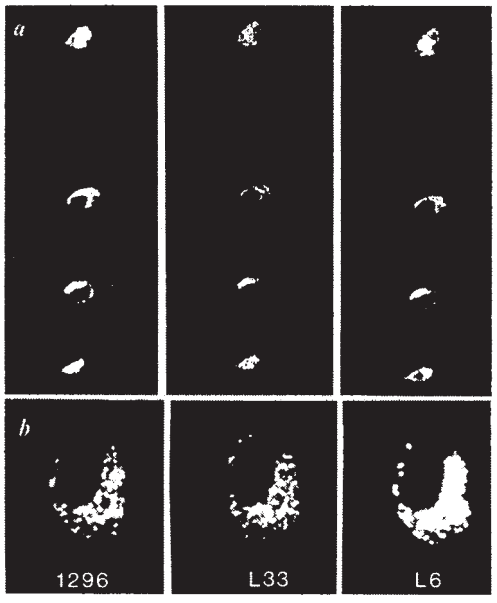

Fig. 2 Hog antral mucosa. *a*, Identical gastrin cells are revealed and *b*, a similar population of granules is demonstrated within a single cell, in serial sections treated with antisera specific for the COOH-terminus of G17 and G34 (1296), the NH_2-terminus of G34 (L33) and intact G17 (L6). Fresh tissue was fixed in buffered formaldehyde, washed in buffer containing 8% sucrose, dehydrated and embedded in resin. Ultrathin sections were etched for 4 min in a saturated solution of sodium hydroxide in ethanol, and subjected to indirect immunocytochemistry (ref. 9), using fluorescein isothiocyanate-conjugated goat anti-rabbit IgG to localise the site of antigen–antibody reaction. Sections were incubated for 65 h in antisera diluted 1 : 200 (1296, L33) or 1 : 600 (L6). Specificity was established by preabsorption of the diluted antisera with excess antigen or related peptide in concentrations up to 25 nmol ml^{-1}. Thus, the reaction with 1296 was abolished after pretreatment with sulphated porcine G17 and a COOH-terminal fragment of human G17 (5–17) but not after absorption with an NH_2-terminal fragment of human G17 (1–13); the reaction with L33 was abolished after absorption with porcine sulphated G34 but not G17, and that with L6 was abolished by treatement with G17 but not 1–13 or 5–17 fragments of G17. *a*, ×550; *b*, ×1,600.

important to note that the antisera were used in immunocytochemical studies at the same dilutions as those used in the analysis of specificity by binding of labelled peptides (Table 1). We therefore attribute immunofluorescence with L33 to G34 or NT G34, that with 1296 to G34 or G17, and that with L6 to G17 alone.

Thus, we conclude that all G-cells which contain G17 also contain either G34 or NT G34, thus excluding the possibility of separate populations of cells containing only G34 or G17. However, the present data do not exclude the possibility of quantitative differences in the relative amounts of G34 and G17 in different cells or in different granules. Previous studies have established that the ratio of G17 to G34 in whole antral mucosa (10–20:1) differs markedly from that in blood (1:2) (ref. 11). In part, the difference may be accounted for by the longer half life of G34, but even so there is relatively more G34 than anticipated, and it seems possible that G34 is released preferentially. This might be a consequence of populations of secretory granules (or separate cells) which, because of incomplete conversion, are relatively rich in G34 and are selectively released. Further analysis of the subcellular distribution of the gastrins is now needed to clarify this possibility.

We thank the MRC for grant support, Professor R. A. Gregory and Dr H. J. Tracy for pure samples of natural porcine G34 and G17, and Elaine Clarke, Carol Higgins and Gavin Laing for technical assistance.

G. J. DOCKRAY

Department of Physiology,

CAMILLE VAILLANT
C. R. HOPKINS

Department of Histology and Cell Biology (Medical), University of Liverpool, Liverpool, UK

Received 20 March; accepted 28 April 1978

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Desensitisation of peripheral photoreceptors shown by blue-induced decrease in transmittance of *Drosophila* rhabdomeres

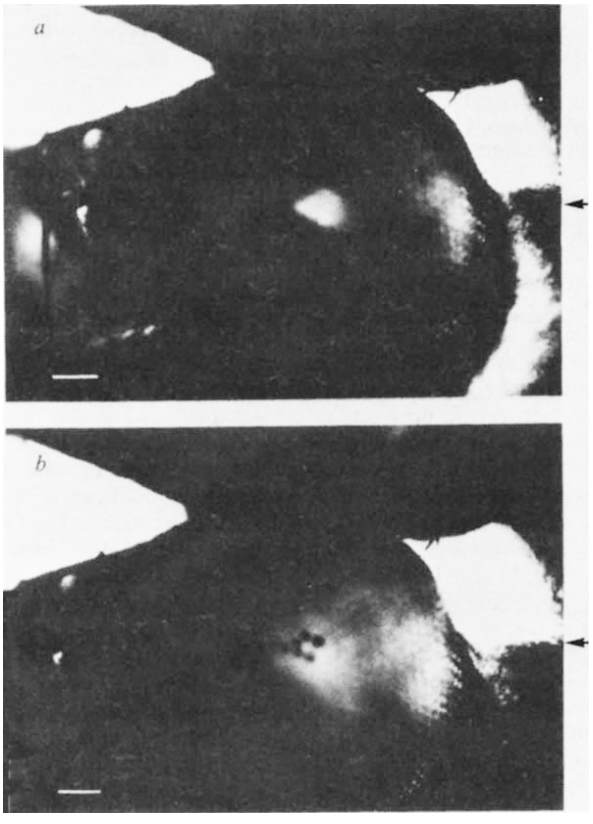
WHILE studying the deep pseudopupil[1,2] of the white-eyed fruitfly (*Drosophila melanogaster*) compound eye, we found that the amount of light transmitted through the peripheral rhabdomeres (Rh_{1-6}) decreases when the eye is illuminated with intense blue light[3]. A similar phenomenon has been independently observed by Cosens and Wright[4]. By using various mutants with defective photoreceptor responses, we examined some possible explanations for this phenomenon and its relationship to the electrophysiological responses of the photoreceptors. The results of these studies are presented here.

The deep pseudopupil (DPP), which is observed with a microscope focused near the centre of curvature of the eye, is the superposition of the magnified ($\sim 10\times$) virtual images of homologous rhabdomeres from many neighbouring ommatidia[1]. The *Drosophila* compound eye consists of about 700 ommatidia, each containing six peripheral and two central retinula cells (photoreceptors). Each retinula cell has a rhabdomere, a specialised membrane structure containing the visual pigment. Because the rhabdomere has a higher refractive index than its surroundings, it works as a waveguide[5]. Thus, the DPP of *Drosophila* consists of seven bright spots, six corresponding to the rhabdomeres (Rh_{1-6}) of the six peripheral retinula cells and one corresponding to the rhabdomeres ($Rh_{7,8}$) of the two central retinula cells arranged end-to-end.

As the rhabdomeres work like optical waveguides, one would expect the DPP of white-eyed *Drosophila melanogaster* to be seven bright spots on a dimmer background. This was indeed the case if the fly had been previously yellow adapted (Fig. 1*a*), but when the eye was illuminated with bright blue light (Corning filter 5–60; $I \sim 5\times10^{15}$ photons $cm^{-2}\,s^{-1}$), the superimposed images of Rh_{1-6} turned darker than the background within a few seconds (Fig. 1*b*), indicating that the light transmitted through these rhabdomeres had decreased. This decrease in transmittance persisted for at least one hour in the dark after a blue stimulus. Only when the eye was illuminated with yellow or longer wavelength light ($\lambda \geq 580$ nm, Ditric 2-cavity filter: $I \sim 3\times10^{15}$ photons $cm^{-2}\,s^{-1}$) did the images of Rh_{1-6} gradually return to their original brightness (Fig. 1*a*).

The visual pigment, rhodopsin (R_{480}), in the peripheral retinula cells of *D. melanogaster* absorbs maximally at about 480 nm and, when exposed to blue light, photoconverts to a thermostable metarhodopsin (M_{580}) absorbing maximally at about 580 nm. Yellow light reconverts M_{580} to R_{480} (refs 6, 7). Thus, the observed change in transmittance through the rhabdomeres caused by blue and yellow stimuli was apparently related to photointerconversion between R_{480} and M_{580}. In fact, the spectral sensitivity for the initiation of DPP darkening peaked at 470–475 nm (Fig. 2), corresponding to the rhodopsin absorption spectrum.

Fig. 1 The eye was illuminated with *a*, yellow light and *b*, blue light. In addition to the bleaching (yellow or blue) light coming from the fibre optics as indicated by the arrows, there was also bright white light coming from the back of the head (antidromic illumination) to provide enough illumination to photograph the deep pseudopupil. (In the figure, the white light would enter the head from the other side of the page.) The pictures were taken with Kodak Tri-X Pan film, and the contrast was enhanced photographically. The scale shown in each figure is 100 μm.



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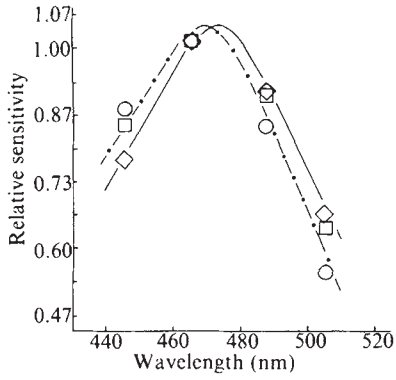


Fig. 2 The spectral sensitivity for inducing DPP darkening was obtained by determining the rate of darkening as a function of wavelength. Sensitivity at a given wavelength is the reciprocal of light intensity needed to darken the DPP at a criterion rate of 1/half-time = 0.3 s^{-1}. Results from three flies (represented by three different symbols) were normalised at 465 nm. Two Dartnall normgrams peaking at 470 nm and 475 nm were plotted as dotted and continuous curves, respectively. The experimental data fitted the normgrams reasonably well.

However, the darkening phenomenon was clearly observable in a class of mutant flies having low levels of M_{580} absorption (about 15% of normal) but not observable in another class of mutant flies having considerably higher M_{580} absorption (up to 70% of normal). These absorption measurements, made in intact animals both in the case of mutant and normal flies, suggest that the darkening of DPP is not directly due to light absorption by M_{580}. Rather, some other process consequent on the photoconversion of R_{480} and M_{580} seems to be involved in the mechanism of the change in transmittance of Rh_{1-6}. This suggestion is strongly supported by the fact that the time course of the transmittance change is very much slower than the pigment conversion. When illuminated with a strobe flash, it took more than 40 ms for the transmittance at 656 nm to change. We know of no reported specific measurements of the time course of $R_{480} \rightarrow M_{580}$ photoconversion; however, it is almost certain to be much less than 1 ms.

Another possible explanation for the DPP darkening is the migration of the ommochrome granules present in the retinula cell. It has been shown that these granules migrate laterally toward or away from the rhabdomere to control the light flux through the rhabdomere[8]. Evidence suggests that ommochrome granule migration is triggered by the receptor potential[8–10]. Many alleles of the mutant *norpA* display no receptor potential[11], and the mutant *trp* displays an abbreviated receptor potential when exposed to a bright stimulus[11,12]. Correspondingly, the *norpA* mutants show no ommochrome granule migration, and the *trp* mutants only a transient one. Nevertheless, the darkening of DPP was not affected by such mutations as *norpA* and *trp*, suggesting that the darkening of DPP is not triggered by the receptor potential. Therefore, it follows that the transmittance change in Rh_{1-6} cannot be due to the migration of ommochrome granules. The above suggestion is strongly supported by the observation that the DPP darkening is observed in white-eyed flies, a strain lacking the ommochrome granules.

In the peripheral photoreceptor of white-eyed *Drosophila*, an intense blue stimulus which converts a substantial amount of R_{480} to M_{580} is known to induce a prolonged depolarising potential which far outlasts the stimulus[13]. This phenomenon has been termed 'prolonged depolarising afterpotential' (PDA). In addition, the blue stimulus desensitises the peripheral photoreceptors, that is, no further responses can be elicited even with high intensity blue stimuli. An intense orange stimulus, which converts M_{580} back to R_{480}, terminates both the PDA and desensitisation of peripheral photoreceptors[13].

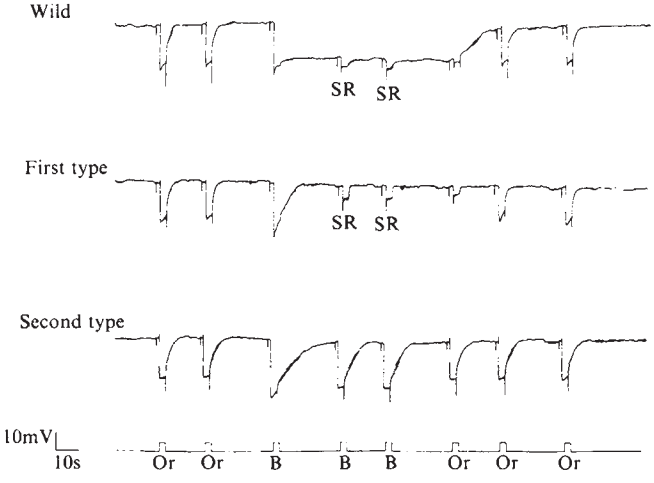
The relationship between the PDA, desensitisation and the darkening of Rh_{1-6} images, which are all induced by blue light and terminated by orange light, was studied by comparing two classes of PDA-defective mutants with wild-type flies. Their electroretinograms (ERG) elicited by blue and orange stimuli are shown in Fig. 3. In wild-type flies, both the PDA and the desensitisation exist. As shown in the top row of Fig. 3, following two orange stimuli, an intense blue stimulus induces both the PDA and desensitisation in the peripheral photoreceptors, so the second and third blue stimuli induce only small responses superimposed on PDA. These superimposed responses have been shown to originate from the central cells, which are not desensitised by blue stimuli[13]. The orange light (sixth stimulus) terminates both the PDA and desensitisation so that the peripheral photoreceptors regain their sensitivities to subsequent stimuli[4,13]. In the first class of mutants (Fig. 3, second row), the first blue stimulus still desensitises the peripheral photoreceptors even though no PDA is visible, and so only the responses originating from the central cells are evoked by the second and third blue flashes. In the second class of mutants (Fig. 3, 3rd row), the responses to the second and third blue flashes are similar to the first blue response, indicating that blue stimuli neither induce a PDA nor desensitise the peripheral photoreceptors.

The darkening of Rh_{1-6} images was present in the mutants of the first class just as in wild type, showing that the darkening of DPP does not depend on the PDA. In the second class of mutants, the darkening phenomenon could not be induced, suggesting that the darkening phenomenon is associated with the desensitisation of peripheral photoreceptors following blue illumination.

Vitamin A deprivation is known to block both the PDA and desensitisation in both wild-type flies[14,15] and mutants of the first class[15]. The darkening of DPP could not be induced in flies deprived of vitamin A, consistent with the suggestion that the darkening of DPP is closely associated with the blue-induced desensitisation in the peripheral photoreceptors.

In conclusion, the blue-induced transmittance decrease in Rh_{1-6} is due neither to direct light absorption by M_{580} nor the migration of ommochrome granules in the retinula cells. The

Fig. 3 ERG responses from the PDA-defective mutants. All of the ERG recordings were obtained from white-eyed flies. Corning filters 5–60 and 2–73 were used for blue (B) and orange (Or) stimuli, respectively. Light intensities were approximately 5×10^{15} and 3×10^{16} photons cm^{-2} s^{-1} for the blue and orange stimuli, respectively. In wild type flies, both the desensitisation and PDA exist. In the first class of PDA-defective mutants, the desensitisation is present even though no PDA is visible, and so only the responses from retinula cells 7 and 8 (SR) are evoked by the second and third blue flashes. In the second class of mutants, the blue stimuli neither induce a PDA nor desensitise the peripheral photoreceptors. A 5-mV calibration pulse precedes every response.



studies of PDA-defective mutant flies and flies deprived of vitamin A suggest that the blue-induced darkening in Rh_{1-6} images is closely associated with the precursor step(s) leading to the desensitisation, which in turn seems to precede the development of the PDA in the peripheral photoreceptors. Therefore, this darkening of Rh_{1-6} images may be a new, noninvasive means for examining the mechanism of desensitisation of peripheral photoreceptors following intense blue illumination. Insofar as the desensitisation is closely associated with both the pigment photoconversion and the PDA, the desensitisation may be an integral component of regulation of photoreceptor membrane permeability by light.

We thank Mrs Marion Pak for editing the manuscript and Ms Sherry Conrad for isolating the PDA-defective mutants. This work was supported by grants from USNIH and NSF.

MEI-VEN C. LO
WILLIAM L. PAK

Department of Biological Sciences,
Purdue University,
West Lafayette, Indiana 47907

Received 6 February; accepted 17 April 1978.

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Pineal eye and behaviour in *Xenopus* tadpoles

THE pineal complex of lower vertebrates contains photoreceptors similar to those in the retina[1]. These photoreceptors are in synaptic contact with ganglion cells whose impulse discharge is decreased by light and enhanced by dimming in most of the animal groups examined (Agnathans, Elasmobranchs, Teleosts and Amphibians)[2]. In Amphibians, pineal photoreceptors have been implicated in control of pigmentation, synchronisation of circadian activity patterns, phototaxis, compass orientation and in the perception of linearly polarised light[3]. However, no simple reflex responses have been reported. It is therefore interesting that in young tadpoles of the clawed toad (*Xenopus laevis*), pineal photoreceptors seem to be responsible for a simple escape response. The evidence presented here indicates that the pineal eye can initiate swimming when the illumination is dimmed.

When a shadow is cast over young larvae of *Xenopus*, some start to swim. This response to general dimming was investigated in embryos removed from their egg membranes and in hatched larvae up to two days old. At each developmental stage[4], 10 animals were placed on the bottom of a shallow white-bottomed dish of water. Before each test they were manoeuvred so that they were lying on the bottom and not hanging from their cement gland mucus, as this has an inhibitory effect on responses[5]. Figure 1 shows the number responding to reducing the illumination at each stage tested. Embryos raised at higher temperatures (20–22°C) respond to dimming one or two stages earlier in development.

The behavioural testing showed that for approximately half a day before and after hatching (between stages 33 and 39) dimming is an effective way to elicit swimming. The location of

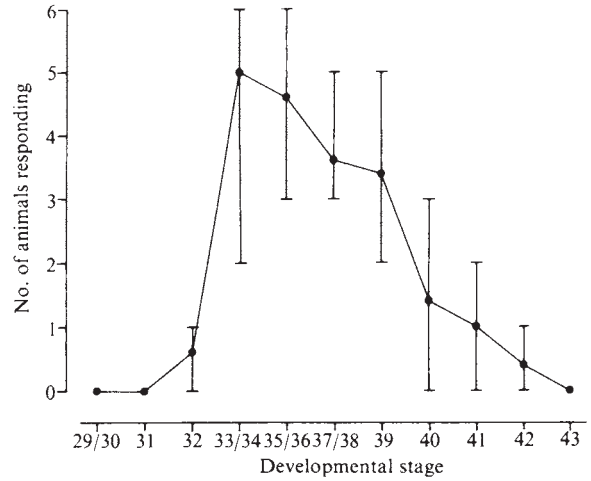

Fig. 1 The number of animals responding to dimming at different developmental stages[4]. The dots indicate averages and the vertical bars the ranges. Dimming was produced by turning off a 60 W light 300 mm above the dish. (Reduction in illumination of about 1,500 lm m^{-2}.) At each stage, 10 animals were tested 5 times to see how many moved or started to swim during 10 s of dim light. A rest of 5 min was given between tests which were carried out at 20–22 °C. Embryos were reared at 18 °C.

the receptors excited by dimming was investigated in stage 37/38 embryos, removed from their egg membranes, paralysed in 10^{-4} M curare, and pinned dorsal side up in a dish of Ringer's solution. The occurrence of 'swimming' activity was monitored by recording from motoneurone axons in the cleft between myotomes using a suction electrode[6]. Dimming the bath illumination evoked a rhythmic motoneurone discharge at swimming frequencies, even when both lateral eyes had been removed (Fig. 2). In this condition, local dimming of the roof of the brain between the eye sockets still evoked swimming but dimming the forebrain or hindbrain had no effect. On the other hand, moving a small spot of light (150 μm in diameter) over the midbrain evoked swimming whenever the area of the pineal was dimmed. To confirm that the pineal was involved, it was exposed by removing overlying skin and cutting through the connective tissue meninges lying above it. With the lateral eyes intact and the pineal exposed to the bathing Ringer's solution, dimming still evoked swimming. However, if the pineal stalk was cut, taking care not to damage the roof of the diencephalon, all response to dimming ceased, although swimming could still be evoked by stroking the skin.

Experiments thus implicated the pineal organ in the embryo's response to dimming. At the stages of development when this response is found, the pineal organ consists of a dorsoventrally flattened vesicle. It joins the brain with a slightly

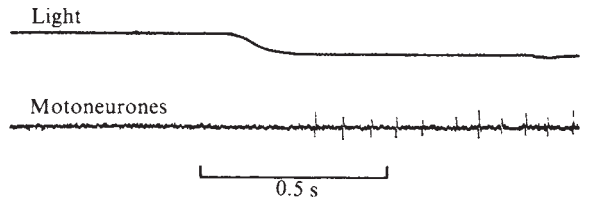

Fig. 2 Initiation of 'swimming' activity in motoneurones of a paralysed, stage 37/38 embryo whose lateral eyes had been removed. Lower trace is recording with a suction electrode (60 μm tip opening) from the left 7th intermyotome region. Upper trace is photocell output to indicate when local illumination of the brain by a light guide was reduced. Recordings were made at 20–22 °C in Ringer with: NaCl, 100 mM; $NaHCO_3$, 20 mM; NaH_2PO_4, 0.1 mM; KCl, 2.5 mM; $CaCl_2$, 1.8 mM at *p*H 7.4 and tubocurarine chloride at 10^{-4} M.

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constricted stalk, has a thin roof just under the developing meninges, and a central cavity in connection with the brain ventricles. Primary photoreceptors with receptor processes lying in this cavity have been described in *Xenopus* embryos as early as stage 35/36 (ref. 7) so recordings were made from stage 37/38 embryos to see if responses to dimming were present. A suction electrode (30 μm tip opening) on the caudal side of the exposed pineal organ recorded an irregular discharge of biphasic impulses of 4.5–7.5 ms duration (Fig. 3), presumably generated by the second-order ganglion cells. The range of amplitudes and waveforms indicated that these impulses came from several separate neurones firing individually at rates of 0.2–2 Hz. Dimming caused a transient increase in the number of impulses recorded, and raising the light intensity caused a brief fall (Figs 3 and 4). After about one minute at any illumination level the discharge rate per second returned to a similar level (Fig. 4). The time constant for

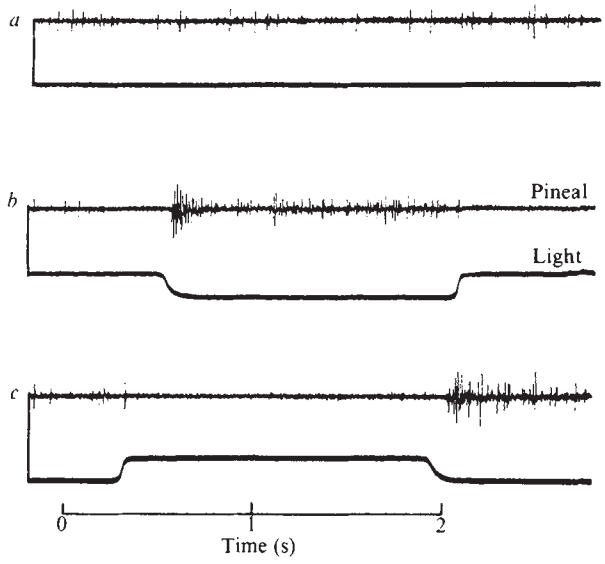

Fig. 3 Recordings of impulse activity from the pineal organ of a stage 37/38 *Xenopus* embryo, showing: *a*, spontaneous discharge in light; *b*, responses to brief dimming; *c*, responses to brief increasing of the illumination. The lower trace moves down as illumination is decreased. Recorded in Ringer without tubocurarine chloride in an isolated head[5].

adaptation after stepwise changes in illumination was between 5 and 10 s. Larger step changes in illumination evoked larger and longer changes in impulse discharge. Resting discharge and similar responses to changes in illumination were still recorded from the pineal organ when its connection to the roof of the brain was cut.

At the stages of development around hatching in *Xenopus* there is no evidence that the lateral eyes can influence swimming behaviour. They probably become functional a day or so after hatching. On the other hand, the pineal organ seems to operate as a third eye. It contains recognisable photoreceptors[7], and, like that of the adult frog[2] is excited by light-dimming. Finally, the pineal organ has to be present for dimming to evoke swimming activity. I therefore propose that in embryo and young larval *Xenopus* (between stages 33/34 and 40 (ref. 4)), pineal photoreceptors are sensitive to dimming, generate an 'off' response in pineal sensory ganglion cells, and can thus initiate swimming by neuronal pathways probably connecting to the hindbrain. The latencies of the pineal impulse discharge and the swimming response are compatible with this proposal. Though there is evidence from fish and amphibians to suggest that pineal photoreceptors could influence phototaxis and swimming activity[3,8–10], a direct role in mediating a shadow response does not seem to have been found elsewhere. Such shadow responses are widely distributed in invertebrate animals; for example, the barnacle withdraws its appendages when shaded[11]. The present evidence suggests that primitively, pineal photoreceptors in the third eye could have mediated similar shadow responses in vertebrates before paired, image forming, lateral eyes had developed. The pineal eye will probably be found to play related roles in other amphibian tadpoles, in fish larvae and perhaps also in some more mature animals.

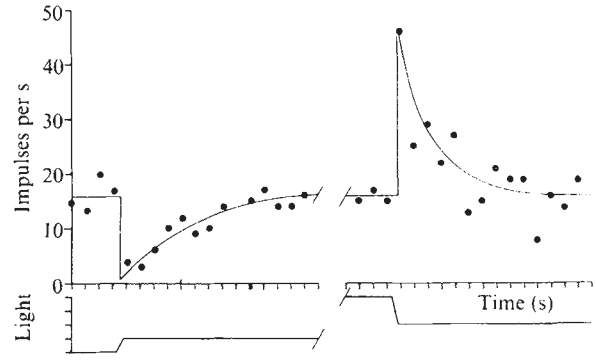

Fig. 4 Effects of changes in illumination on the impulse discharge from the pineal organ. Each point indicates the number of impulses per 1-s period which exceeded an arbitrary voltage threshold. Before each change in the illumination, the preparation was in constant conditions for 2 min. Note that with different initial levels of illumination the number of impulses per second is similar.

ALAN ROBERTS

Department of Zoology,
University of Bristol,
Bristol, UK

Received 13 February; accepted 28 April 1978.

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Evolutionary and dietary aspects of salivary basic (Pb) and post Pb (PPb) proteins in anthropoid primates

IN this paper we detail the evolution of the biochemically and immunologically related Pb and PPb proteins in the saliva of anthropoid primates, using electrophorectic and immunologic criteria. Whereas the PPb protein evolved in hominoids after their divergence from cercopithecoids and before the hylobatid-pongid/hominid divergence, the Pb proteins evolved earlier and are found in cercopithecoids and some platyrrhines. We further show that there is a good relationship between the electrophoretically determined forms of the Pb proteins in salivas of anthropoid primates and the type of diet they consume as well as with certain aspects of their dental structure. Electrophoretically complex forms of the Pb proteins are associated with a predominantly frugivorous-folivorous diet, whereas simpler forms are seen when the diet is predominantly insectivorous.

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Previous studies[1-4] established the genetic and biochemical relationships of the human Pb proteins, a set of unique low molecular weight parotid basic salivary proteins that show genetic polymorphism in American Blacks. By electrophoretic criteria, there are four human Pb proteins (a, b, d and e) in the common Pb 1-1 genetic type (Fig. 1 *A*, channels 1 and 6).

Monospecific precipitating antibody prepared in rabbits to these human Pb proteins reacted with the Pb proteins as well as with another protein in human saliva. The second protein, termed the post Pb (PPb) protein (named for its elution properties on Bio Gel P-10 chromatography), was found to be immunologically identical to the Pb proteins by gel diffusion and immunoelectrophoresis[5]. Genetic and biochemical evidence indicated that the PPb protein and the Pb proteins, although closely related, are products of separate gene loci[5]. The function of the Pb and PPb proteins is unknown[6].

Our data suggested that the PPb protein may have evolved in hominoids sometime after their divergence from cercopithecoids[5]. The squirrel monkey did not show basic proteins resembling those of the human as assessed by electrophoresis, suggesting that the Pb proteins may have evolved in catarrhines sometime after their divergence from platyrrhines[2]. However, this conclusion was considered tentative since only one platyrrhine species was studied and immunologic tests were not done.

The electrophoretic patterns of the salivary basic proteins from the various species are shown in Fig. 1. Only the distal portions of the gels containing the most basic proteins of rapid mobilities are shown. For reference, the positions of the human Pb proteins a, b, d, e and the PPb protein are shown on all gels (gel *A*, channels 1 and 6; *B*, 1 and 6; *C*, 1; and *D*, 1 and 8). Pongid and hylobatid samples include the chimpanzee (*Pan troglodytes*; *C*4), gorilla (*P. gorilla*; *C*2), orang-utan (*Pongo pygmaeus*; *C*3), and gibbon (*Hylobates lar*; *C*5). The cercopithecoid samples include the the rhesus (*Macaca mulatta*; *C*6 and *D*2), crab-eating (*M. fascicularis*; *C*7), pig tail (*M. nemestria*; *D*3), and stump tail (*M. arctoides*; *D*4) macaques. The platyrrhine samples include the owl (*Aotus trivirgatus*; *A*, 2 and 3), capuchin (*Cebus albifrons*; *A*, 4 and 5) and spider (*Ateles geoffroyi*; *B*, 4 and 5) monkeys. In summary, basic salivary proteins of man and several species of greater and lesser apes, Old World monkeys and New World monkeys show an overall resemblance with a multibanded pattern usually represented by 4–6 bands. Similar results have been previously seen with the baboon (*Papio cynocephalus*) and mandrill (*Mandrillus sphinx*)[5]. However, two platyrrhine species, including the cotton-top tamarin (*Saguinus oedipus*; *B*2) and the squirrel monkey (*Saimiri sciureus*; *B*3 and *D*5) as well as the random bred domestic dog (*D*6) and Sprague–Dawley rat (*D*7) do not show the typical multibanded patterns in the basic protein region of the gel. They show a fainter single band in the case of the two platyrrhine species and even slower migrating bands in the case of the dog and rat that bear no resemblance to the patterns found in most primates.

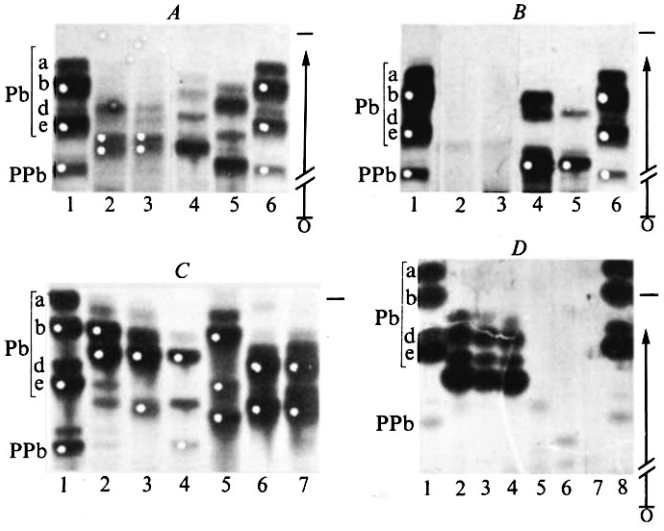


Fig. 1 Electrophoretic patterns of primate salivary basic proteins. *A*, *B* and *C* are distal portions of acid–urea polyacrylamide gels and *D* the distal portion of an acid–urea starch gel[2] stained for arginine-rich proteins[14]. Human parotid saliva samples were collected using the Lashley cup placed over Stenson's duct in the mouth. Nonhuman primates' saliva samples were collected from the cheek pouch near the parotid duct opening. Saliva flow was stimulated in the human using sour lemon candy placed on the tongue and in the nonhuman primates following injection of the cholinergic stimulant, carbachol. An inhibitor of proteolysis (Trasylol) 500 U ml^{-1} was added to most samples. Each channel represents a sample from a different animal except for the human samples that are duplicated on gels *A*, *B*, and *D*. Gel *A*: channels 1 and 6 are human, 2 and 3 are owl monkeys, 4 and 5 are capuchin monkeys. Gel *B*: channels 1 and 6 are human, 2 is tamarin, 3 is squirrel monkey, 4 and 5 are spider monkeys. Gel *C*: channel 1 is human, 2 is gorilla, 3 is orang-utan, 4 is chimpanzee, 5 is gibbon, 6 is rhesus macaque, 7 is crab-eating macaque. Gel *D*: channels 1 and 8 are human, 2 is rhesus macaque, 3 is pig tail macaque, 4 is stump tail macaque, 5 is squirrel monkey, 6 is dog, and 7 is rat.

In Ouchterlony double diffusion tests several of these basic salivary proteins from the various nonhuman primate species gave precipitin reactions with the antiserum to human Pb proteins indicating their immunologic and biochemical relatedness to the human Pb proteins. This was determined by cutting out plugs of polyacrylamide gel containing the separated basic proteins and placing them in wells in Agarose adjacent to wells containing antiserum to the human Pb proteins. In Fig. 1 the proteins showing precipitin reactions to the antiserum are indicated by white spots on the separated proteins of polyacrylamide gels *A*, *B* and *C*. Some of the components failed to give precipitin lines, but this is more likely to reflect differences in sensitivity to the antiserum prepared against human Pb proteins than the presence of unrelated basic proteins. Ouchterlony analysis using the same antiserum showed only a single line of partial or complete identity when saliva from man and a nonhuman primate, either gorilla, chimpanzee, orang-utan, gibbon, rhesus macaque or squirrel monkey, were compared.

Immunoelectrophoretic comparisons of salivas from different species of primates are shown in Fig. 2. A comparison is made on each slide between man (above) and a nonhuman primate species (below). The antiserum was prepared in rabbits against human Pb proteins[5]. A double arc pattern with anionic and cationic components joined by a line of immunologic identity is shown in man (all slides), chimpanzee (slide *E*) and gibbon (slide *D*). Saliva of the gorilla and orang-utan gave similar patterns (not shown). On the other hand, saliva of the squirrel monkey (slide *A*), crab-eating macaque (slide *B*), and rhesus macaque (slide *C*), showed only single components, cationic in the latter two and overlying the origin in the former. Single cationic components were also seen on immunoelectrophoresis of salivas of the owl and spider monkeys, and stump tail and pig tail macaques (not shown). Precipitin reactions could not be obtained against salivas of the capuchin monkey, tamarin, dog or rat. As expected, because of increasing phylogenetic distance, the strongest precipitin reactions occurred with the human saliva and became progressively weaker in the sequence of pongids, hylobatids, cercopithecoids, and platyrrhines.

To summarise and interpret the immunologic data, we found an anionic component in the saliva of hominoids but not in the salivas of cercopithecoids and platyrrhines. This anionic component represents the PPb protein (the cationic component represents the Pb proteins). Therefore, we conclude, in confirmation and extension of our previous work[5], that the PPb protein evolved in hominoids relatively recently after their

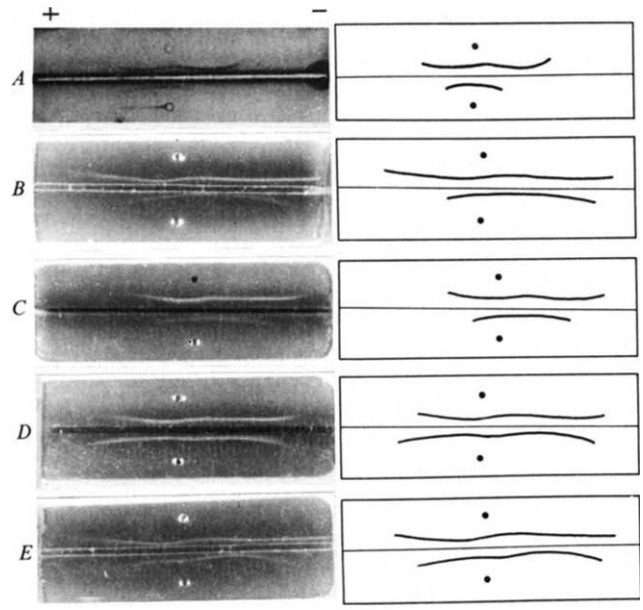
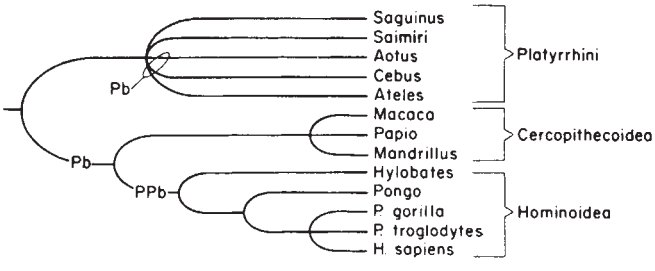


Fig. 2 Immunoelectrophoresis of primate salivas using rabbit antiserum to human Pb proteins[5]. The gels are prepared on slides and contain 0.8% agarose in 0.05 M sodium barbital/hydrochloric acid buffer, *p*H 8.6. Slides *B* and *E*, *C* and *D*, and *A* were electrophoresed on different trays respectively. The saliva samples are concentrated tenfold by lyophilisation and reconstitution in gel buffer. The antiserum is concentrated fivefold by dialysis against 15% (w/v) polyethylene glycol (molecular weight 20,000) in 0.9% (w/v) saline. On each slide, human samples are on the top and nonhuman primate samples below. Slide *A*: squirrel monkey. Slide *B*: Crab-eating macaque. Slide *C*: rhesus macaque. Slide *D*: gibbon. Slide *E*: chimpanzee.

divergence from cercopithecoids and prior to the hylobatid-pongid/hominid divergence (Fig. 3). The gene coding for the PPb proteins may have evolved from the gene coding for the Pb proteins by a process of gene duplication. By illustration and comparison, there are numerous examples of variations in the numbers of haemoglobin α chain genes occurring within the various primate species[7].

The multibanded patterns seen after electrophoresis of Pb proteins in hominoids, cercopithecoids and platyrrhines, with exception of the tamarin and squirrel monkey, show remarkable similarities in overall appearance. The callithrichids, represented by the cotton-top tamarin, are believed by some to be the most primitive anthropoid family[8,9]. Thus, the divergence in electrophoretic pattern of Pb proteins of the tamarin compared to the other species, and the failure of its saliva to give precipitin reactions with the antiserum to human Pb proteins could be explained on the basis of phylogenetic distance from man. Such an explanation, however, is in conflict with immunological findings on albumins and transferrins which indicate monophyletic origins for anthropoids[10]. Furthermore, the divergent electrophoretic pattern of the Pb proteins in the squirrel monkey militates against such an explanation.

Fig. 3 The postulated evolution in anthropoid primates of typical multibanded forms of Pb proteins and the related PPb protein. The arrangement of species from top to bottom tends to follow a dietary gradient from predominantly insectivorous (*Saguinus* and *Saimiri*) to predominantly frugivorous-folivorous (all others except man, which is omnivorous).



A better explanation may involve a relationship between the evolution of Pb proteins, diet and body size. In primates, increasing body size is associated with changing metabolic regimens as reflected by a dietary gradient from insectivorous to frugivorous-folivorous[11]. Of the species investigated in this study, the tamarin and squirrel monkey have the smallest body size which is correlated with a smaller frugivorous-folivorous portion of the diet than in larger sized species. Since fruits and leaves consist largely of carbohydrates, we postulate that the evolution of Pb proteins might relate to an increased amount of dietary carbohydrates in larger species. More specifically, we suggest that components of the Pb proteins may interact with the tooth enamel in such a manner as to make it more resistant against microinjuries (caries) associated with the amount of carbohydrate or varying texture of the diets. In support of this hypothesis, other workers have shown certain basic proteins with *p*I values as high as 10 to adsorb on to the hydroxyapatite surfaces from the parotid saliva[12].

Our argument that the evolution of Pb proteins may relate to a dietary gradient is supported by observations on the evolution of dental cingula. Dental cingula are ridges of enamel at the base of the tooth crown and evolve probably in response to functional requirements of mastication and thus are related to diet. Among platyrrhines, the callithrichids and squirrel monkey have well developed cingula (in particular, greatly developed mandibular cingula) which seem to be correlated with a predominantly insectivorous diet[13]. In contrast, predominantly frugivorous-folivorous species have less developed cingula. This suggests that in anthropoid primates both the evolution of Pb proteins and the reduction of dental cingula may be viewed as a response to a shift from a highly insectivorous to a predominantly frugivorous-folivorous diet. It is also suggested that Pb proteins may have evolved in parallel fashion in platyrrhines and catarrhines (Fig. 3). Another equally tenable hypothesis is that the Pb proteins arose in protoanthropoids and were lost in the insectivorous *Saguinus* and *Saimiri*. To test these hypotheses on the evolution and biological role of Pb proteins, further studies on insectivorous and frugivorous-folivorous prosimians are planned.

We thank M. Goodman, W. G. Kinzey, and J. E. Cronin for constructive criticism and Judy Innvaer and Renate Ciampi for technical assistance. We also thank D. Florence, W. Houser, B. Swenson, T. Kawakami, and P. K. Sehgal for primate saliva samples. This study was supported by grant DEO 3658-13 from the National Institutes of Dental Research.

EDWIN A. AZEN

Department of Medicine,
University of Wisconsin

WALTER LEUTENEGGER

Department of Anthropology,
University of Wisconsin,
Madison, Wisconsin 53706

ERWIN H. PETERS

Faculty of Medicine,
Division of Medical Biochemistry,
University of Calgary,
Calgary, Alberta, Canada

Received 24 February; accepted 17 April 1978.

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Analysis of virion RNA segments and polypeptides of influenza A virus recombinants of defined virulence

THE genome of the avian influenza virus A/fowl plague/27 (A/FPV) and other influenza A virsuses consists of eight species of single-stranded RNA[1–3]. Each of these RNAs represents the gene for one virus-coded polypeptide, and RNA–polypeptide assignments have been made for several influenza A virus strains[3,4]. Furthermore, in suitable conditions the RNA fragments of different influenza A viruses migrate at different rates in polyacrylamide slab gels containing 6M urea and differences in the polypeptide migration rates can be measured[3–5]. Thus, in recombinant influenza viruses, which are commonly used as candidate attenuated vaccine strains (see ref. 6 for review), the parental origin of each RNA gene can now be established. We report here the first analysis of the genomes of a set of human influenza virus recombinants of defined virulence. By analysis of the parental origins of the recombinants' genomes we attempted to answer questions concerning the relationship of possible gene groups or constellations to virus virulence and attenuation.

The recombinant series used in this study was originally isolated and characterised biologically by McCahon and Schild[9]. Clinical studies were carried out using volunteers as described by Beare and Reed[10]. One parent A/PR/8/34 (HON1) virus had a high yield of virus haemagglutinin in embryonated hens' eggs and was attenuated in virulence for man[7,8]; the other parent A/England/69 (H3N2) was virulent for man and provided a low yield of haemagglutinin in eggs. The recombinants were either virulent or attenuated for man and, with the exception of clone 7, gave a high yield of haemagglutinin in eggs. The viruses were cloned by terminal dilution and titration in 10-day-old embryonated hens' eggs. For polypeptide or RNA analysis, virus was purified by differential and rate zonal centrifugation as described previously[11]. Virus concentrates of 15 mg ml^{-1} protein were stored at −70 °C. RNA was extracted from two preparations of each virus (see Table 1).

Table 1 Parental origin of the genes of six influenza recombinants

Gene no. and assignment	Recombinants virulent for man		Recombinants attenuated for man			
	clone 6	clone 7	clone 64c	clone 64d	clone 31	clone 64b
1 (P protein)	P	E	E	P	P	P
2 (P protein)	P	E	E	P	P	P
3 (P protein)	P	E	P	P	P	P
4 (HA)	E	E	E	E	E	P
5 (NP)	P	E	P	P	?	P
6 (NA)	E	E	E	E	P	E
8 (NS1)	P	E	P	E	P	P

P, gene originating from the A/PR/8/34 parent virus. E, gene originating from the A/England/69 parent virus. Genes 5 and 6 of A/PR/8/34 encode NP and NA, respectively, and genes 5 and 6 of A/Eng/69 encode NA and NP, respectively. All data have been converted to the A/PR/8/34 numbering. HA, haemagglutinin; NS1, nonstructural protein number 1; NP, nucleoprotein; NA, neuraminidase. Virus RNA was extracted and fractionated by electrophoresis through 2.6% and 2.8% polyacrylamide slab gels containing 6M urea as described previously[12]. These gels were 22 cm long × 15 cm × 1.5 mm and were prepared as described by Palese and Schulman[3]. 2–5 μg of each RNA preparation were loaded in 1-cm slots and electrophoresis carried out for 27 h at 110 V, at room temperature and with tank buffer recirculation. The gel slabs were then stained for 30 min with 0.2% toluidine blue in 40% methoxyethanol and destained with several changes of 30% methoxyethanol until the stained RNA bands became visible.

Analysis of migration patterns in polyacrylamide gels of toluidine blue-stained RNA extracted from the recombinants indicated the presence of eight bands in each virus. In the recombinants, RNAs coding for seven of the eight virus polypeptides could be identified by migration rate differences as originating from either the A/PR/8/34 or the A/England/69 parent viruses (Table 1). Gene 7, coding for virus matrix protein, from both parents migrated at a similar rate in these conditions of electrophoresis and could not be unambiguously distinguished. Also gene 5 of clone 31 migrated with the corresponding A/PR/8/34 RNA band in 2.6% acrylamide gels but in a position between the two parental RNA bands in 2.8% gels and so could not be clearly assigned. However, polypeptide analysis (see below) indicated comigration with A/PR/8/34 nucleoprotein. Further, analysis of clone 31 gene 5 RNA by digestion with T_1 RNase and fractionation on two-dimensional polyacrylamide gels demonstrated A/PR/8/34 origin. Examination of the gene composition of the four attenuated recombinants (Table 1) showed that they had various gene combinations from the two parents. No particular constellation or group of genes was characteristic of all viruses which could have resulted in the attenuation property except that all the attenuated recombinants possessed genes 3 and 5 derived from the A/PR/8/34 parent. However, the virulent clone 6 virus also possessed those two genes from A/PR/8/34 virus. Of particular interest was the gene composition of clones 7 and 6, both virulent viruses for man. Clone 7 was shown to be identical to the A/England/69 parent in all seven genes examined by the above technique. Clone 6, in contrast, had received at least five of the eight genes from the avirulent A/PR/8/34 parent and yet was virulent for man.

Confirmation of the parental origin of the NP, HA and NS1 genes established above by direct analysis of virus RNA, was obtained by investigation of migration rate differences of the virus polypeptides induced in Vero cells and pulse labelled with ^{35}S-methionine[13,14]. Clear differences (Fig. 1) were detected in the rates of migration of the HA, NP and NS1 polypeptides. The NS1 polypeptide migrated in these gels as a split double band moving slightly faster than the M protein. The HA migrated as a diffuse band above the NP polypeptide. We have so far been unable to distinguish clear migration rate differences in the M polypeptides between the two parents used in these experiments.

Virulence of influenza viruses is an important biological property: the molecular mechanism for virulence is not known, although there is evidence to indicate a multigenic nature for pathogenicity[15,16]. Cleavage of virus HA may be an important factor, and experiments with A/FPV have suggested that gene P3 may control virus host range and hence contribute to virus virulence[13]. Studies in chickens with recombinants of the avian influenza virus A/Turkey/Ontario/66 (Hav5Nav6) have suggested that the genetic basis of virulence is multigenic and linked with genes 3 and 5 (ref. 17). Scholtissek *et al.*[18] investigated the pathogenicity of a series of recombinants of fowl plague virus for chickens and concluded that attenuation depended on the genes which were exchanged as well as on the influenza strain from which the new gene was derived, but they detected no specific gene constellation in attenuated recombinants. In our study, all the recombinant virus strains which

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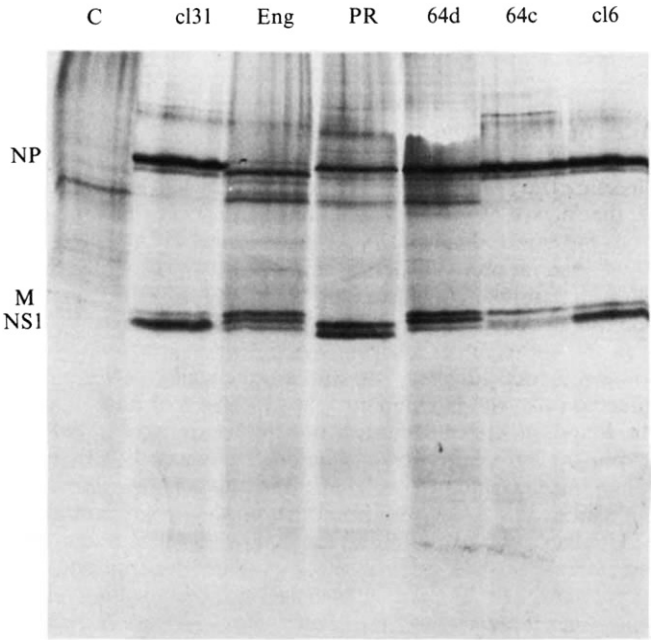

Fig. 1 Differences in migration rates of ^{35}S-methionine-labelled polypeptides of A/PR/8 and A/England/69 parent viruses. NP, nucleoprotein polypeptide; M, matrix polypeptide; NS1, non-structural polypeptide 1; Eng, A/England/69 (H3N2); PR, A/PR/8/34 (HON1). 64d, 64c, cl 6, and cl 31, recombinant clones as described in text and Table 1. For analysis of ^{35}S-methionine-labelled virus polypeptides, Vero cells (a continuous line of monkey kidney cells) were infected with allantoic fluid suspensions of virus to give a multiplicity of approximately 10 plaque-forming units per cell. After 6 h or 18 h incubation at 35 °C the cells were pulsed for 30 min with 20μCi per 60-mm Petri dish in 0.2 ml Geys medium of methionine (specific activity 1,040 Ci mmol^{-1}, Radiochemical Centre, Amersham). The cells were then washed and lysed with 0.2 ml of a mixture of 2% w/v sodium dodecyl sulphate (SDS) and 0.6% v/v β-mercapto-ethanol, heated at 100 °C for 2 min, and 20-μl volumes layered onto slab gels of 17.5% polyacrylamide using high resolution discontinuous buffers, and electrophoresed for 18 h at 40 mA (ref. 12). Gel slabs were dried and autoradiographed by procedures described previously[14]. Examination of the autoradiograph of the dried gel slab indicates migration rate differences in NP and NS1 polypeptides. Band splitting is noted with the NS1 polypeptide but the band pattern of clone 64d, for example, is similar to that of the A/England/69 parent; the split NS1 bands of clones 64c and 6 resemble those of the A/PR/8 parent.

gave a high yield of haemagglutinin in eggs contained gene 3 and gene 5 from A/PR/8/34. Thus, these genes correlated with the ability of the recombinants to grow to high titre in eggs like the A/PR/8/34 parent. This association could have other origins; for instance, recombinants with A/PR/8/34 genes other than these two might be nonviable without P1 and NP from A/PR/8/34 virus. Nevertheless, identification of recombinants with genes 3 and 5 from the high yielding A/PR/8/34 parent will be useful for the selection of suitable viruses for the production of inactivated influenza vaccine. Interestingly, clone 7 consisted of at least seven genes from the A/England/69 parent and therefore may be the result of mutation of this parent rather than a reassorted form. This virus had an ability to grow in eggs which was intermediate to that of the two parental viruses. Recombinant clone 6 had at least five genes originating from the A/PR/8/34 parent and only the genes coding for HA and NA glycoproteins originated from the virulent A/England/69 parent. Nevertheless, the recombinant was virulent for man. In addition, earlier studies in volunteers indicated that the recombinant X-31 virus which has six of eight genes from the A/PR/8/34 parent and only genes coding for HA and NA from the virulent A/HK/1/68 parent (J. Schulman, personal communication) was partially virulent for man[7]. Thus, even the level of genetic characterisation achieved in this study does not allow prediction of the state of attenuation for man of particular recombinant clones, and mutational events during laboratory manipulation, in addition to reassortment, may contribute to this.

We thank R. Newman for preparation of purified influenza viruses, Terry Corcoran for technical assistance and B. Watts for photography. The volunteer experiments referred to conformed to MRC standards for investigations on human subjects and were part of long-term studies approved by the Ethical Committee of the Clinical Research Centre, Northwick Park.

J. S. OXFORD

D. J. MCGEOCH*

G. C. SCHILD

A. S. BEARE†

Division of Virology,
National Institute for Biological Standards and Control,
London NW3, UK

Received 21 February; accepted 12 April 1978.

*Present address: MRC Virology Unit, Institute of Virology, Church Street, Glasgow, UK.
†Present address: MRC Common Cold Research Unit, Harvard Hospital, Salisbury, Wiltshire, UK.

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Nuclear conversion of microinjected avian leukosis virion RNA into an envelope-glycoprotein messenger

IN eukaryotes, cellular[1–4] as well as viral[5–7] messenger RNA molecules may contain nucleotide sequences complementary to discontinuous regions of DNA. This is apparently the case with subgenomic avian RNA tumour virus mRNAs. In these viruses the entire genetic information is contained on a 30–40S[8,9] virion RNA molecule which is transcribed from a DNA provirus integrated into the host chromosomes[10]. Subgenomic viral mRNAs contain only genes located in the 3′ half of the virion molecule[11–15], but contain 5′ terminal nucleotide sequences (ref. 13; W. S. Hayward and W. Haseltine, personal communication) and oligonucleotides[16] apparently identical to those at the 5′ terminus of the larger virion molecule. As has

0028–0836/78/0273—0779$01.00

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been suggested for other mRNAs, subgenomic RNA tumour virus mRNAs might be derived from a larger precursor[17] by processing involving cleavage and subsequent ligation, resulting in the elimination of internal nucleotides from the precursor[5,18]. The data do not rule out the possibility, however, that subgenomic mRNAs are produced by transcription of discontinuous DNA sequences within the integrated provirus; or even that they result from transcription of a subgenomic provirus. The biological experiments reported here support the hypothesis that the envelope-glycoprotein messenger of the avian leukosis virus RAV-2 (Rous associated virus-2) can be derived from a larger precursor, and indicate that the virion RNA itself can function as the precursor.

Within RAV-2-infected cells viral envelope-glycoprotein genetic sequences are expressed in full-genomic 35S mRNA as well as in a 21S mRNA[12–14]. Previous experiments using the technique of microinjection have shown that the 21S RNA functions as the messenger for envelope-glycoprotein (*env* mRNA). To demonstrate this, RNA was microinjected into the cytoplasm of cells transformed by the envelope-deficient Bryan strain of Rous sarcoma virus [RSV(−) cells]. The ability of the injected RNA to complement the Bryan RSV deficiency by directing the synthesis of envelope-glycoprotein was indicated by the release of focus forming units (FFU) of infectious RSV from the injected cells. 21S RNA from RAV-2-infected cells functioned far more efficiently as *env* mRNA than 35S cellular RNA[19]. A similar conclusion has been reached by others using amphibian oocyte[20] and cell-free[21] translation systems.

In this study 35S RNA from RAV-2 virus particles, which is similar if not identical to 35S viral-specific RNA within infected cells, was injected into the cytoplasm of RSV(−) cells. As found previously[19] it functioned poorly as *env* mRNA within the cytoplasm and promoted the release of few FFU. When injected into the nuclei of these cells, on the other hand, the virion 35S RNA promoted the production of numerous FFU (Table 1). Apparently the *env* gene within the full-genomic virion molecule was made available for translation by a nuclear event(s). The properties of the resulting active messenger molecule are not known. However, the fact that it was able to function effectively within a living cell to direct the synthesis of a biologically active molecule suggests that it is similar to the *env* mRNA produced by virus-infected cells.

To rule out simple cleavage of virion RNA as the mechanism of its nuclear conversion to active envelope messenger, the *env* mRNA activity of fragments of virion RNA was studied. 35S RAV-2 virion RNA was purified twice by velocity sedimentation and then subjected to cleavage by treatment with a mild base. Fragmented RNA was then fractionated by velocity sedimentation and fragments in various size fractions ranging from 18S to 30S were microinjected into the cytoplasm of RSV(−) cells. Similarly, fragments of virion RNA were selected on oligo(dT)-cellulose (for molecules containing poly(A), and therefore the 3′ proximal envelope genetic information)[11,15] and various size fractions were microinjected into the cytoplasm of RSV(−) cells. No FFU were released by cells receiving any of these injections (Table 1). In addition, oligo(dT)-cellulose selected or unselected fragments from 24S to 30S were injected into the nuclei of RSV(−) cells without subsequent appearance of FFU. If nonspecific hydrolysis of virion RNA were entirely responsible for its conversion within the nucleus to active envelope messenger, the fragments injected into the cytoplasm might have exhibited envelope-messenger activity. Even if specific nuclear cleavage were responsible for the activation of virion 35S RNA, it is possible that the 24–30S fragments would have been properly cleaved following injection into the nucleus to yield active molecules. While these negative results are only suggestive, they agree with the idea that active *env* mRNA must be produced from full-genomic RNA by a processing sequence involving specific internal cleavage and splicing of the 5′ nucleotide sequences to the 5′ end of the cleavage product.

To estimate the time required for the nuclear conversion of virion RNA into active envelope messenger molecules, amounts of infectious virus released at 3-h intervals following 270 intranuclear injections of twice-purified virion 35S RNA were determined. The resulting profile of total virus release (Fig. 1) is very similar to that observed following cytoplasmic injection of 21S *env* mRNA obtained from RAV-2-infected cells[19]. A 3-h lag in infectious virus production was followed by a sharp increase in the rate of production to a peak at 9 h following injection, which was followed by a rapid decline. This result indicated that the rate-limiting steps in the production of active envelope-glycoprotein are the same whether *env* mRNA is injected into the cytoplasm or its presumptive precursor is injected into the nucleus. The nuclear conversion of virion 35S RNA into active envelope messenger must, therefore, be rapid in relation to the cytoplasmic processes involved: translation, glycosylation and association of the envelope glycoprotein

Table 1 Comparison of the envelope messenger activities of RAV-2 virion RNA following cytoplasmic and nuclear injection into RSV(−) cells

RNA injected	RNA concentration (mg ml^{-1})	No. of injections	Injection site	FFU released
35S*	2.0	200	Nucleus	236
35S*	2.0	200	Cytoplasm	3
21S†	0.1	450	Cytoplasm	848
21S fragment‡	0.6	450	Cytoplasm	0

RNA was prepared from RAV-2 virus particles, fractionated by velocity sedimentation and microinjected into RSV(−) cells as previously described[19]. Culture fluids were collected for 18 h following injection and assayed for focus forming virus as described[19]. Nuclear injections were performed so that little or no alteration in the size of the injected nucleus was observed. The fact that injection had taken place was indicated by the lightened phase-contrast appearance of the nuclei. The effect of these injections on the cells was determined by phase-contrast observations for up to 20 min following injection. Temporary granularisation of the cytoplasm was not uncommon, but permanent damage to injected cells was generally associated only with inadvertently large injections. Nuclear injections were estimated visually to be approximately one-third to one-fourth the size of cytoplasmic injections (which were similar in size to those measured in HeLa cells to be 1.0×10^{-10} ml per injection[29]).

* 35S RNA was obtained from RAV-2 virus particles after one fractionation of RNA by velocity sedimentation as described in the text.

† 21S RNA was obtained from RAV-2 virus particles. The RNA was prepared and fractionated by velocity sedimentation as described above. RNA fractions corresponding to 18–23S were combined and subjected to two additional purifications by velocity sedimentation to approximate the treatment of the RNA fragments described below. The extremely high activity of this RNA preparation as *env* mRNA is consistent with recent observations which suggest that 21S *env* mRNA from RAV-2 infected cells is encapsulated into virus particles (ref. 19; our unpublished observations; personal communication, W. S. Hayward).

‡ 21S fragments were prepared from 35S virion RNA which had been fractionated twice by velocity sedimentation to remove any contaminating 21S *env* mRNA. This 35S RNA was then shown to express no *env* mRNA activity following cytoplasmic injection into RSV(−) cells, but high activity following nuclear injection. Fragments were obtained by treatment of RNA dissolved in 0.05 M Na_2CO_3 at 50 °C for 4 min. The fragmented RNA was then fractionated by velocity sedimentation for 6.5 h as described previously and the 18–23S fraction was injected for this experiment. In other experiments (not described in the above table) higher molecular weight fragments were injected into the cytoplasm and nuclei of RSV(−) cells. For these experiments fragments in various size fractions from 24 to 30S at concentrations from 0.1 to 1.0 mg ml^{-1} were injected into RSV(−) cells. In addition fragments of 35S virion RNA were selected on oligo(dT)-cellulose for poly(A)-containing fragments which were similarly tested. No FFU were released following any of these injections.

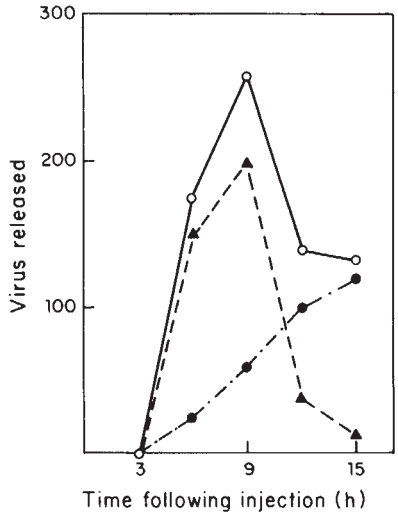

Fig. 1 Time-course of infectious virus release following intranuclear injection of virion RNA. RAV-2 35S virion RNA was purified twice by velocity sedimentation to remove any contaminating 21S RNA which would express *env* mRNA activity (see legend to Table 1). This RNA (1.0 mg ml^{-1}) was injected into the nucleus of 270 RSV(−) cells. The numbers of focus-forming virus (●), RAV-2 (▲), and total virus (○) released following these injections were determined from culture fluids collected at 3-h intervals from the injected cells. The titre of focus-forming virus was determined by focus assay on normal chick cells as previously described[19]. The number of RAV-2 (helper virus) released was estimated by dilution analysis. Nine plates of normal chick cells were each infected with a solution containing 1% of the original culture fluid. The infected cells were transferred after 2 d and subcultured with RSV(−) cells. Culture fluids from these cultures were collected on the fourth day and assayed for the production of focus-forming virus (which would indicate the presence of helper virus in the original inoculum). The proportion of the nine cultures which became the producers of focus-forming virus was used to estimate the titre of RAV-2. The total virus released represents the sum of focus-forming virus and RAV-2. In the control culture which received 270 intracytoplasmic injections of the 35S virion RNA, no infectious virus was observed within 18 h following injection. It is important to note that infectious virus production early after injection must be due to the *env* mRNA activity of the injected RNA rather than to infection of RSV(−) cells by newly produced RAV-2 since no *env* mRNA could be produced as the result of any viral infection for at least 8–10 h following injection[30].

produced with virus particles. The observation that the active messenger molecule derived from virion 35S RNA exhibited translation kinetics and stability within RSV(−) cells indistinguishable from those of 21S *env* mRNA from infected cells strongly suggests that the two molecules are very similar if not identical. In the control culture of this experiment which received 270 cytoplasmic injections of virion 35S RNA no infectious virus were produced within 18 h after injection.

When the amounts of infectious virus released from cells following nuclear injections of virion 35S RNA are compared to the amounts released following cytoplasmic injection of *env* mRNA[19] (taking into consideration that nuclear injections are estimated by visual comparison to be from one-third to one-fourth the size of cytoplasmic injections) it is apparent that 35S virion RNA injected into the nucleus of RSV(−) cells is at least as effective in complementing their envelope deficiency and promoting infectious virus production as the most active *env* mRNA preparations injected into the cytoplasm. Apparently the nuclear conversion of virion RNA into active envelope messenger molecules is efficient (although it does not seem to go to completion as described below).

These data along with other studies indicate that 35S viral-specific RNA is a versatile molecule. It can serve as the messenger for viral core proteins[22,23], and polymerase[24–26], can be encapsulated into the virion, and from the work described here can apparently be converted into envelope-glycoprotein messenger. This would suggest that full-genomic viral RNA within the nucleus is only partially converted into *env* mRNA as shown schematically in Fig. 2. This conclusion is tentatively supported by the observation that the titre of RAV-2 was greater than that of FFU within the first 9 h following intranuclear injection of RAV-2 virion 35S RNA into RSV(−) cells (Fig. 1). We believe this result is best explained by the passage of up to one-half of the injected RNA out of the nuclei without being processed. (The 35S virion RNA thus entering the cytoplasm would then be encapsulated into virions to produce infectious RAV-2 particles; see Fig. 2 legend.)

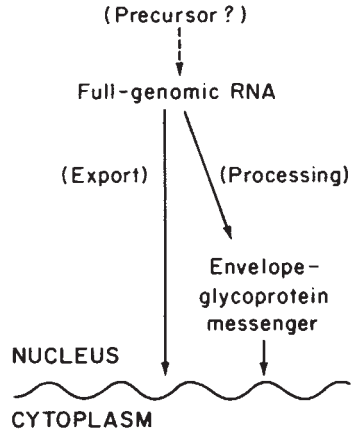

Fig. 2 Partial nuclear conversion of viral RNA into envelope messenger. The evidence presented in this paper suggests that full-genomic, 35S viral RNA serves as the nuclear precursor for *env* mRNA. This RNA also functions directly as a cytoplasmic messenger and as viral genome. These facts along with the suggestive experimental evidence discussed below indicate that full-genomic viral RNA may be only partially converted within the nucleus to *env* mRNA while the rest is transported to the cytoplasm without alteration. This hypothesis predicts that the ratio of full-genomic to subgenomic viral RNA within the cytoplasm can be controlled at the level of nuclear processing. It is not known if a precursor for the 35S viral molecule exists. Suggestive experimental evidence also indicates that virion 35S RNA is only partially processed within the nucleus. From the ratio of RAV-2 to FFU produced following intranuclear injection of virion 35S RNA into RSV(−) cells, it is apparent that at least a fivefold excess of RAV-2 to RSV 35S viral RNA must have existed within the cytoplasm of injected cells between 3 and 6 h following injection. This would require that approximately 50% of the injected RNA had escaped unaltered from the nucleus to the cytoplasm. In separate experiments fluorescent-labelled proteins, known to be restricted in their passage across the nuclear envelope, were injected into the nuclei of HeLa cells. From subsequent fluorescent microscopic observations it was apparent that only approximately 10% of the cellular fluorescence was localised within the cytoplasm even 6 h following injection, while the rest had remained within the nuclei (D. W. S. and V. G. Allfrey, unpublished observations). Therefore, it is considered extremely unlikely that the number of RAV-2 RNA molecules within the cytoplasm of injected RSV(−) cells could have been inadvertently injected there or could have escaped from the injected nucleus as a result of the injection process. It is possible however, that the sudden placement of a large number of molecules simultaneously into a nucleus would lead to irregular processing events. Nevertheless, this data is taken as suggestive evidence in support of the hypothesis that full-genomic viral RNA is only partially processed to *env* mRNA within the nucleus.

The suggestion that only a fraction of the full-genomic viral RNA within the nucleus is processed leads to the possibility that the ratio of subgenomic to full-genomic viral RNA within the cytoplasm can be controlled at the level of nuclear processing. This might explain why mammalian cells (presumably with different nuclear processing characteristics than avian cells) express only subgenomic viral-specific RNA within the cytoplasm following infection with avian sarcoma viruses, while both subgenomic and full-genomic viral RNA is present within their nuclei[27,28]. The mammalian cells apparently process all viral RNA within the nucleus.

If, as suggested, RAV-2 virion RNA can serve as the messenger or as the nuclear precursor to the messenger of each viral gene in addition to its ability to function as viral genome, it is possible that virion RNA injected into the nucleus of uninfected cells could promote virus release from them. To test this hypothesis purified 35S RAV-2 virion RNA (1.0 mg ml^{-1}) was injected into the nucleus of 600 chick embryo fibroblasts which expressed no endogenous viral functions. As predicted, the injected cells released approximately 10 infectious RAV-2 viruses within 9 h after injection. If this RNA were injected into the cytoplasm of the uninfected cells, however, comparable virus production was not observed unless 21S *env* mRNA was simultaneously injected. In addition to confirming that virion 35S RNA can provide all messenger functions required for virus production once it is exposed to a nuclear processing activity, this result indicates that the nuclear activity required to convert virion RNA into active envelope messenger is present in uninfected cells.

The data reported above provide evidence that the precursor for RAV-2 envelope-glycoprotein messenger RNA is similar to virion RNA. The strength of this evidence rests on the fact that living cells performed the critical chemical processes involved in the analysis: nuclear activation of virion RNA, translation of the resulting envelope messenger molecule, and assay of the biologically active envelope-glycoprotein produced. The fastidious nature of the injected cell is indicated by the fact that virion RNA injected into the cytoplasm was less than 0.1% as active as the same RNA injected into the nuclei. Furthermore, fragments of virion RNA have been totally inactive as envelope messenger in studies conducted to date. It is likely under these conditions that the results described represent the normal characteristics of the cells and molecules studied. The most important result of this work, however, may be that it identifies a readily obtainable nuclear precursor for an mRNA which can be sensitively and rigorously assayed.

We thank Grace Minasian for technical assistance and V. G. Allfrey for use of equipment. D.W.S. is supported by an NIH fellowship. This work was supported by grants CA14935 from the US NCI and VC-225 from the American Cancer Society.

DENNIS W. STACEY

HIDESABURO HANAFUSA

The Rockefeller University, New York, New York 10021

Received 13 March; accepted 17 April 1978.

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Rapid transfer of non-histone chromosomal proteins to the nucleus of living cells

CHROMATIN of eukaryotic cells is composed of DNA, histones and non-histone chromosomal proteins. Histones are generally thought to be nonspecific gene repressors and components maintaining the structure of chromatin. On the other hand, recent reports suggest that non-histone chromosomal proteins activate regions of repressed DNA by their association with histones[1]. This idea was mainly derived from studies at the transcriptional level with reconstituted chromatin *in vitro*[2]. The ultimate function of biological substances in cells might be determined by injecting these substances directly into living cells. Accordingly, we recently developed a method for introducing macromolecules into culture cells using erythrocyte ghosts (ghost fusion method)[3]; the method is based on virus-induced cell fusion of resealed erythrocyte ghosts containing the substance with recipient cells. To obtain clues to the function of non-histone chromosomal proteins inside cells, we have now used this method to introduce iodine-labelled non-histone chromosomal proteins from rat liver into mouse cells and then studied distribution of these proteins in the recipient cells. We found that they were rapidly transferred to the cell nuclei.

^{125}I-labelled non-histone chromosomal proteins were introduced into Ehrlich ascites tumour cells. After various times of incubation at 37°C, the cells were lysed and separated into cytoplasmic and nuclear fractions and the amount of radioactivity in each fraction was determined. As shown in Table 1, the non-histone chromosomal proteins introduced into the cytoplasm were rapidly transferred to the nuclei of recipient cells (within 30 min, see legend to Table 1), and the maximal amount of radioactivity was found at the start of incubation (0 h). The amount of radioactivity in the nuclei did not decrease appreciably during subsequent incubation for up to 24 h, but the amount of radioactivity in the cytoplasm decreased rapidly to about 20% of the initial amount. Therefore, the amount of radioactivity in the nuclei as a percentage of the total radioactivity increased from 22% to 49% during incubation for 24 h.

The chromatin was then isolated from the nuclei to find whether the radioactivity in the nuclear fraction was bound to recipient chromatin. About 60% of the radioactivity in the nuclei was found in the chromatin fraction at all times examined (Table 1). When about 2×10^7 nuclei (2×10^7 nuclei per ml) isolated from these cells by the same method were

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Table 1 Changes in radioactivity of iodinated non-histone chromosomal proteins and BSA in nuclear, cytoplasmic and chromatin fractions of recipient cells during incubation

		Radioactivity in nuclear and cytoplasmic fractions			Radioactivity in chromatin and nucleoplasmic fractions		
	Incubation (h)	Nucleus (N)	Cytoplasm (C)	$\frac{N}{N+C}$(%)	Chromatin (Ch)	Nucleoplasm (Nu)	$\frac{Ch}{Ch+Nu}$(%)
Non-histone chromosomal proteins	0	36,200	145,000	22	19,000	14,300	57
	4	33,100	51,800	39	19,200	10,500	66
	24	28,200	29,100	49	16,300	9,100	64
BSA	0	5,400	73,200	6.9			
	26	1,300	13,600	8.7			

Non-histone chromosomal proteins were isolated from male rat liver by a slight modification of the method of van den Broek *et al.*[13]. In brief, minced liver was homogenised in a Waring blender in 0.25 M sucrose, 3.3 mM $CaCl_2$ and then centrifuged at low speed. The crude pellet of nuclei was washed 3 times with the same solution and finally purified by centrifugation in 2.3 M sucrose at 19,000 r.p.m. for 1 h. The purified nuclei obtained were apparently free from cytoplasmic contamination when examined by electron microscope. These nuclei were homogenised in 0.01 M Tris-HCl (*p*H 8.0) and centrifuged at 10,000 r.p.m. for 15 min. This procedure was repeated three times. The resulting gelatinous precipitate was dissolved in 3 M NaCl, 5 M urea, 10 mM Tris-HCl (*p*H 8.3) and stirred at 0°C for 1 h. The protein fraction was separated from undissolved chromatin by centrifugation at 64,000 r.p.m. for 40 h according to the method of Stein *et al.*[14]. Non-histone chromosomal proteins were separated from histones on a Bio-Rex 70 column. These proteins were iodinated with ^{125}I by the procedure of Greenwood *et al.*[15]. The specific activities of the resulting non-histone chromosomal proteins and BSA were 4×10^6 c.p.m. μg^{-1} and 5×10^6 c.p.m. μg^{-1}, respectively, and more than 95% of the radioactivity could be precipitated with 5% trichloroacetic acid. The method for introducing these proteins into recipient cells was described elsewhere[3]. In brief, iodinated proteins were introduced into human erythrocyte ghosts by dialysing a mixture of erythrocytes and the proteins against hypotonic solution. The resulting ghosts were washed several times by centrifugation at 2,800 r.p.m. for 8 min to remove free proteins not entrapped in the ghosts. Then a 10% suspension of the ghosts, a 10% suspension of Ehrlich ascites tumour cells and 500 haemagglutination units of UV-inactivated HVJ (Sendai virus) were mixed in a ratio of 2:3:5 and shaken for 20 min at 0°C and then for 30 min in a water-bath at 37°C. In these conditions about 80% of the recipient cells were found to have fused with loaded ghosts when examined by fluorescent microscope using antiserum against human erythrocyte membranes. After fusion, the preparation was subjected to repeated centrifugation with culture medium at 0°C until contamination with free ghosts was judged to be less than 0.5% under a phase contrast microscope. The cells were then cultured in Eagle's minimal essential medium containing 10% calf serum. The start of cultivation was taken as 0 h. At the times indicated, 2×10^7 cells were collected by centrifugation and lysed with NP-40 at a final concentration of 0.25% in the presence of protease inhibitor (phenylmethylsulphonylfluoride). The nuclear fraction was separated from the supernatant cytoplasmic fraction by centrifugation, and chromatin was isolated from the nuclei by the method of Stein *et al.*[14]. The recovery of radioactivity in the chromatin and nucleoplasmic fraction from nuclei was 88–92%. Radioactivity was determined with a Packard 3002 gamma spectrometer. The values given are means of two experiments.

incubated with iodinated non-histone chromosomal proteins for 15 min at 0°C, 1.7% of the radioactivity became associated with the nuclei and of this only 8.0% was bound to chromatin. This result excludes the possibility that non-histone chromosomal proteins are adsorbed adventitiously to the nuclear fraction.

In contrast, when bovine serum albumin (BSA) was introduced into the cells, it mainly remained in the cytoplasm where it was rapidly degraded (Table 1), and the small amount of BSA transferred to the nuclei was degraded at the same rate as that in the cytoplasm. Thus, the ratio of radioactivity in the nucleus to that in the cytoplasm remained constant during incubation. In recent micro-injection studies using fluorescein isothiocyanate-conjugated BSA we found that fluorescence was mainly detected in the cytoplasm (unpublished data), a finding consistent with the results in Table 1.

The molecular profiles of non-histone chromosomal proteins obtained from chromatin and the cytoplasmic fraction of recipient cells were studied by SDS–polyacrylamide gel electrophoresis. As shown in Fig. 1*a*, *b*, *c*, the positions of the main peaks of chromatin fractions were similar and the profiles resembled that of the original iodinated non-histone chromosomal protein sample. In contrast, the profiles of cytoplasmic fractions differed markedly from those of the chromatin fractions, a single prominent peak corresponding to protein of molecular weight ~40,000 being present from the start of incubation (Fig. 1*d*, *e*, *f*). It is uncertain whether this protein(s) is a degradation product(s) of larger non-histone proteins or protein(s) which remained in the cytoplasm without being transferred to the nucleus, but from the initial amount of non-histone chromosomal proteins introduced it seems likely that the protein(s) is a mixture of degradation products and remaining protein(s) at least at 0 h. The fact that the molecular profile of the chromatin differed from that of the cytoplasm, even at the start of incubation, suggests that there was no exchange of introduced non-histone chromosomal proteins between these two fractions during the incubation. It has been reported that contractile proteins, such as myosin, actin and tropomyosin, are included in the non-histone chromosomal protein fraction[4] and that their molecular weights are similar to those of the protein(s) remaining in the cytoplasm. The protein(s) obtained from the cytoplasm of recipient cells after culture for 4 h, however, was not precipitated with rabbit skeletal muscle myosin.

After introduction of BSA, the electrophoretic pattern of the cytoplasm of recipient cells at all times examined consistently showed a single sharp peak corresponding to protein of approximate molecular weight 68,000, although the height of this peak decreased during incubation (Fig. 1*g*, *h*). No similar peak was detected in the electrophoretic pattern of the nuclei and the radioactivity in the nuclei was low.

The rapid degradation of non-histone chromosomal proteins and BSA in the cytoplasm of recipient cells may reflect the natural turnover of these proteins in the original cells, because it is generally accepted that acidic proteins turn over more rapidly than neutral or basic proteins[5]. Alternatively, their turnover rate may be an unnatural phenomenon due to their iodination, denaturation during preparation and unusual location after their artificial introduction, because unusual proteins, such as mutant proteins, are reported to be degraded more rapidly than native proteins[6].

Our results show that non-histone chromosomal proteins were transferred within 30 min to the nucleus where they existed as a stable form, but serum proteins, such as BSA, were hardly transferred to the nucleus, remaining in the most part in the cytoplasm where they were degraded.

Bonner has shown that histones injected into *Xenopus* eggs by micropipette were transferred preferentially to the nucleus[7]

and that they bound to chromosomes of recipient cells in the subsequent metaphase stage[8]. Gurdon showed that when nuclei from HeLa cells were injected into *Xenopus* oocytes, nuclear proteins synthesised by the oocytes migrated to the transplanted HeLa nuclei[9]. It has also been reported that nuclear proteins, such as non-histone chromosomal proteins and histones, are transferred rapidly to the nucleus after their synthesis on ribosomes in the cytoplasm[10]. In heterokaryon produced by cell fusion, nuclear proteins intermigrate from one nucleus to another[11]. These findings suggest that there is some machinery in the cytoplasm by which nuclear proteins are transferred selectively from the cytoplasm to the nucleus.

Studies on cell-free systems suggest that non-histone chromosomal proteins are regulatory factors for gene expression. In somatic cell genetics, changes of marker phenotype often occur after heterokaryon or hybrid cell formation[12]. The most likely explanation of these phenomena is that some regulatory factor(s) from one parent cell acts on the chromosome(s) of another parent cell. Our studies suggest that the biological functions of non-histone chromosomal proteins may be analysed by introducing purified preparations of these proteins into living cells.

Crystallised BSA was purchased from Sigma.

MASARU YAMAIZUMI

TSUYOSHI UCHIDA

YOSHIO OKADA

Research Institute for Microbial Diseases, Osaka University, Yamada-Kami, Suita, Osaka, Japan

MITSURU FURUSAWA

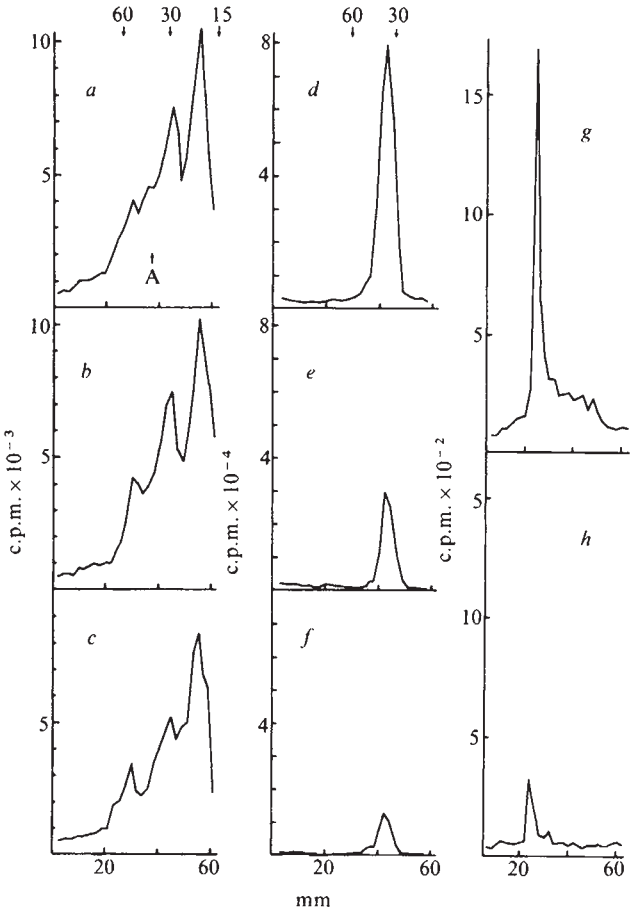
Faculty of Science, Osaka City University, Sugimotocho, Sumiyoshi-ku, Osaka, Japan

HIROMI MITSUI

Tokyo Metropolitan Institute of Medical Science, Honkomagome, Bunkyo-ku, Tokyo, Japan

Received 11 August 1977; accepted 24 April 1978

Fig. 1 Changes of the SDS–polyacrylamide gel electrophoretic patterns of introduced non-histone chromosomal proteins and BSA in recipient cells during incubation. Chromatin and the cytoplasmic fractions were prepared as described in Table 1 and analysed on 0.1% SDS, 7.5% polyacrylamide gel. The ^{125}I-radioactivity in 2-mm slices of the gels was counted. Molecular weights were estimated by comparison of positions with those of proteins of known molecular weight on a parallel gel. The arrows indicate the positions of proteins of molecular weights 1.5×10^4, 3×10^4 and 6×10^4, respectively. Arrow A indicates the position of the peak protein which appeared in the cytoplasmic fraction (Fig. 1*d*, *e*, *f*) when tested by co-electrophoresis of chromatin and cytoplasmic fraction. Non-histone chromosomal proteins: *a*, 0 h, in chromatin; *b*, 4 h, in chromatin; *c*, 24 h, in chromatin; *d*, 0 h, in cytoplasm; *e*, 4 h, in cytoplasm; *f*, 24 h, in cytoplasm. BSA: *g*, 0 h, in cytoplasm; *h*, 26 h, in cytoplasm.



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Photochemical *cis–trans* isomerisation of bovine rhodopsin at liquid helium temperatures

RHODOPSIN is the visual pigment in the disk membranes of vertebrate rod photoreceptors. It consists of a chromophore, 11-*cis*-retinal, covalently bound in the form of a protonated Schiff base to an ε-amino group of a lysine in its apoprotein opsin. The primary photochemical event in visual excitation involves the formation of a species known as bathorhodopsin[1] which has been detected at room temperature[2] and at 4 K (ref. 3). The rhodopsin–bathorhodopsin transformation has been thought to involve a photochemical 11-*cis*→all-*trans* isomerisation; however, this conclusion has recently been questioned[2–5] primarily because bathorhodopsin is formed within a few picoseconds at room temperature[2] and in 36 ps at 4 K (ref. 3), and because of the large isotope effect involved in its formation[3]. In this letter, we show on the basis of resonance

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Raman experiments that isorhodopsin (the artificial pigment with 9-*cis*-retinal as its chromophore) is formed photochemically from rhodopsin at liquid helium temperatures. All models for the primary event other than isomerisation about the 11–12 double bond are found to be inconsistent with this result. We present a specific model (see also ref. 6) for the formation of bathorhodopsin which accounts for all the available data.

The alternative models, not involving photochemical *cis–trans* isomerisation, which have been suggested for the primary event include (1) proton transfer from a ring proton to the opsin[3,4], (2) photoinduced electron transfer from the chromophore to the protein[5], and (3) proton tunnelling near the Schiff base linkage[3]. A number of arguments render all of these specific models unlikely[6,7] but the strongest argument is the one on which the suggestion of a photochemical *cis–trans* isomerisation was originally made[1,8]. Bathorhodopsin is formed photochemically from either rhodopsin or isorhodopsin; and at 77 K a photostationary state between the three species is obtained. The kinetic scheme is given by rhodopsin ($11\text{-}cis \xrightarrow{h\nu}$ bathorhodopsin $\xrightarrow{h\nu}$ isorhpdopsin (9-*cis*) so that bathorhodopsin is a common intermediate between pigments based on two different *cis* isomers and thus must itself contain a 'transoid' chromophore.

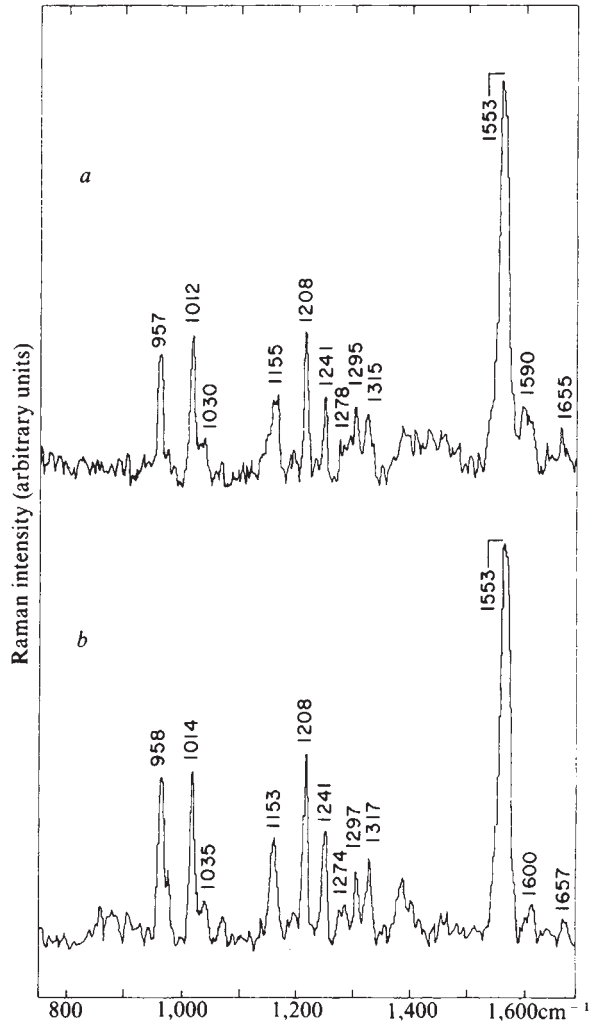
Models for the primary event not involving a *cis–trans* isomerisation cannot account for the photochemical behaviour at 77 K. The only attempt to incorporate isorhodopsin into a specific model was made by van der Meer *et al.*[4] who proposed that bathorhodopsin is a mixture of two spectroscopically indistinguishable species that are in thermal equilibrium. An 11-*cis* conformer is suggested as the photoproduct of rhodopsin with a 9-*cis* conformer being formed from isorhodopsin. Since the models of van der Meer *et al.*[4] and of Peters *et al.*[3] predict that bathorhodopsin is a retro-type structure where double and single bonds have switched relative to the parent pigment, thermal equilibration involves only the simultaneous isomerisation of two essential single bonds. Using the barrier height to isomerisation of one single bond in butadiene of 5.0 kcal mol^{-1} (ref. 9) as an estimate for the barriers involved in the pigment, and a frequency factor of 10^{13}, then thermal equilibration at 77 K should take about 1 min (see below), well within the time it takes to achieve a photostationary state. However, at liquid helium temperatures thermal isomerisation is no longer a possibility. For this reason it is of considerable interest to determine whether isorhodopsin can be formed from rhodopsin in these conditions.

Yoshizawa and coworkers[10] have studied the photostationary state formed from rhodopsin at 4 K. Isorhodopsin was detected on the basis of a resolution of photostationary state spectra into contributions from individual components. This procedure has been used successfully at higher temperatures[1], but it is subject to uncertainties because absorption maxima are not particularly sensitive to isomeric form and because there are always difficulties in the analysis of multicomponent samples. In contrast, the Raman spectrum is an extremely sensitive probe of isomer composition[11], and for this reason a resonance Raman study of the photostationary state formed from rhodopsin at liquid helium temperatures was undertaken.

Figure 1*a* shows the resonance Raman spectrum at a sample temperature of 5.5 K and Fig. 1*b* the Raman results at a sample temperature of 88 K. The irradiating light frequency was chosen as 568.2 nm as the rhodopsin sample becomes completely converted to isorhodopsin at this wavelength at 80 K (ref. 13). It is important to emphasise that the Raman spectra at liquid nitrogen temperature (Fig. 1*b*) have been shown to be essentially identical to those measured in regenerated samples of 9-*cis* retinal and opsin[14], that is, chemically produced isorhodopsin. Moreover, the Raman spectral similarities of 11-*cis* protonated Schiff bases in solution to that of rhodopsin, and 9-*cis* protonated Schiff bases in solution to that of isorhodopsin, prove that the conformation of the chromophores in opsin are essentially 11-*cis* and 9-*cis* for rhodopsin and isorhodopsin respectively[14]. Thus, it has been established that isomerisation about two bonds occurs in the rhodopsin → bathorhodopsin → isorhodopsin transition. The identical resonance Raman results obtained at 88 K (Fig. 1*b*) and at 5.5 K (Fig. 1*a*) then prove that isomerisation also occurs at liquid helium temperatures.

It is a simple matter to estimate an upper limit for the barrier height involved in the rhodopsin–bathorhodopsin transformation. Using the standard expression $k = A\exp(-E/RT)$, a frequency factor A of 10^{13} and an upper limit for k defined by the measurement time of approximately 4,000 s, then an upper limit of $E = 0.18$ kcal mol^{-1} is obtained for the barrier height. This is an order of magnitude smaller than the barrier to isomerisation about the single bond in butadiene[9]. It is clear that thermal isomerisation about single bonds as suggested by van der Meer *et al.*[4] is unrealistic.

Fig. 1 Rod outer segment membrane fragments were isolated from dark-adapted bovine retinal essentially as described previously[12]. Solubilised rhodopsin was obtained by extracting the membrane fragments in 0.75% lauryldimethylamine oxide and 100 mM phosphate buffer (*p*H 7). The Raman apparatus and dewar system were as described previously[13]. The exciting laser irradiation was the 568.2 nm line of a model 52 Coherent Radiation krypton laser at 1.5 mW. *a*, The rhodopsin sample was cooled to and maintained at 5.5 K in the dark and then irradiated. *b*, A separate sample was cooled to and maintained at 88 K in the dark and then irradiated.



Our results demonstrate then that bathorhodopsin cannot be composed of two isomers in thermal equilibrium. Thus the only plausible mechanism for the rhodopsin $\xrightarrow{h\nu}$ bathorhodopsin $\xrightarrow{h\nu}$ isorhodopsin interconversion is an 11-*cis* $\xrightarrow{h\nu}$ all-*trans* $\xrightarrow{h\nu}$ 9-*cis* photoisomerisation. (Note the possibility of two hypothetical forms of bathorhodopsin that are interconverted photochemically making the rhodopsin–isorhodopsin conversion a three-photon process. There is no evidence for this from the studies of Yoshizawa and Wald[1], but it probably cannot be ruled out with certainty. However, one of the major objections to *cis–trans* isomerisation as the primary event is based on the unlikelihood of its occurring at low temperatures[3], so invoking a photochemical two-bond isomerisation at 5 K merely adds complication without alleviating this difficulty.) As kinetic studies have shown that both the 11-*cis* → all-*trans* (ref. 2) and 9-*cis* → all-*trans* (ref. 15) steps occur in picoseconds or less, it is apparent that photochemical *cis-trans* isomerisation can take place in picoseconds and also can occur at liquid helium temperatures. There is little or no barrier to the process in the pigment as has been shown previously on the basis of a photochemical analysis[6,7]. Since protonated Schiff bases in solution do not isomerise either efficiently[7] or so quickly[5], the apoprotein opsin has apparently been designed to catalyse photoisomerisation.

Recently Peters *et al.*[3] found that the formation time of bathorhodopsin is significantly increased in rhodopsin samples suspended in D_2O and concluded on the basis of this and other evidence that the primary event in vision involves a proton transfer rather than a *cis–trans* isomerisation. Since we have shown here that photoisomerisation does occur, it is important to find alternative explanations for their data which do strongly suggest that a hydrogen transfer step is somehow associated with the formation of bathorhodopsin.

One possibility is that isomerisation is complete within the resolution time of their experiment (6 ps) at all temperatures down to 4 K. If so, then the transient whose decay at 570 nm is slowed down by deuterium substitution and low temperatures would be a ground state species rather than an excited state as suggested by Peters *et al.*[3]. A plausible model for the transient might be based on the interaction between the protonated nitrogen and its counter-ion. In the excited state there is a shift of electron density of the chromophore towards the nitrogen[16] and as a result its *p*K should be significantly increased[17]. It is expected then that the Schiff base proton would accompany the nitrogen during isomerisation, leaving it temporarily at least, without a counter-ion. The formation of new hydrogen bonds or salt bridges in the vicinity of the chromophore would then follow isomerisation and could easily involve small barriers for proton translocation of the type detected by Peters *et al.*[3]. In this model the decay of the transient at 570 nm corresponds to a ground state process in which the protein–chromophore complex relaxes to a stable structure after isomerisation. It is extremely unlikely that the chromophore of bathorhodopsin has the same conformation as the all-*trans* Schiff base of retinal free in solution. Indeed, the low frequency Raman lines of bathorhodopsin, first observed by Oseroff and Callender[13], suggest a distorted conformation[18] resulting from the inability of the *trans* chromophore to accommodate itself to the opsin binding site. Twisting about single bonds, which are the internal degrees of freedom of greatest flexibility, seems the most likely possibility.

An extremely rapid isomerisation would also be consistent with the failure of Peters *et al.* to detect a repopulation of the rhodopsin ground state[3]. There is considerable evidence that the rhodopsin and bathorhodopsin ground states are populated from a common point on the excited state potential surface,[6,7] with relative rates of 1:2. Thus the two processes should be complete in less than 6 ps. However, while the opsin must relax to accommodate the isomerised chromophore of bathorhodopsin, repopulation of the rhodopsin ground state would presumably involve no changes in the opsin and thus no measurable transients.

This work was supported by grants from the US NSF (PCM 75-03020 and PCM 77-06728), the Research Corporation, and BHE-PSC City University research award programme.

B. ATON
R. H. CALLENDER

Department of Physics,
The City College of The City University of New York,
New York, New York 10031

B. HONIG*

Department of Biological Sciences,
Columbia University,
New York, New York 10027

Received 6 February; accepted 17 April 1978.

* Permanent address: Department of Physical Chemistry, The Hebrew University, Jerusalem, Israel.

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Erratum

In the letter 'Seroepidemiologic assessment of feline leukaemia virus infection risk for man' by J. M. Krakower and S. A. Aaronson, *Nature* **273**, 463, in paragraph 2 line 21, 'is likely to be a significant' should read 'is unlikely to be a significant'.

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reviews

How Nature works

Kenneth Mellanby

Why Big Fierce Animals are Rare: An Ecologist's Perspective. By Paul Colinvaux. Pp.236. (Princeton University Press: New Brunswick, New Jersey, 1978.) $9.50.

It is an unusual pleasure to find a popular book with "ecology" in its title which is actually about the subject. Professor Colinvaux makes his views clear in his introduction when he writes: "Ecology is not the science of pollution, nor is it environmental science. Still less is it a science of doom". To him, as to me, ecology is about the relationships between organisms, (animals, plants and micro-organisms) and their environment. Professor Colinvaux considers that the true roots of ecology are in the writings of Charles Darwin, who was primarily concerned to show how the world worked. If ecological studies help us to prevent this process from grinding to a halt, and if they show us how to conserve our resources or to fight pollution, so much the better. But these admirable developments are manifestations of serendipity and not a necessary extension of the science.

The important questions posed, and at least partially answered, by this book are: "Why are there so many different kinds of plants and animals? Why are some common but others rare? Why are some large and others small?" We are introduced to the concept of the niche, the habitat and the ecosystem, words with precise meaning often muddled even by professional scientists. Early on in his text, Colinvaux states that every species has a breeding strategy which, operating through natural selection, leaves the largest possible number of surviving offspring. The tactics in breeding, however, differ from species to species. For instance, some birds lay only one egg each year, others have large clutches; but as a rule both have populations which remain more or less the same year after year. The reasons seem to be made clear, though I think that we may sometimes be made to think that a description of a phenomenon is the same as an explanation of why it operates. This is something for the reader to decide.

The question implied by the title—that is, why are big fierce animals rare?—is answered simply and convincingly. Large carnivores need a lot of food; they convert the flesh they eat even more inefficiently than their herbivorous prey converts the grass on which it grazes, so the world can only support small numbers. Large herbivores, being lower down the food chain, can exist in larger numbers, and their very size allows them to escape from all but the largest predators. The huge baleen whales, living on tiny plankton, do not really fit into the general picture—but then they are not fierce.

However, this book is much more than a discussion about animal numbers and energetics. It is a vivid picture of how the natural world works. It stresses how this is constantly changing, with an ordered succession of types of vegetation and of the animals related to that vegetation.

We learn why the sea is blue—because it is largely an unproductive desert. Thus, the potential value of the oceans to feed the growing human population is small. The way our present atmosphere arose and is maintained is discussed, and our adaptation to air with one-fifth part of oxygen, four-fifths of nitrogen and a tiny fraction of carbon dioxide (on which all life depends) is considered.

Professor Colinvaux deals firmly with the common belief of many so-called ecologists that Lake Erie is dead, and with the other even commoner misconception that eutrophication is a synonym for pollution. He explains the natural succession of inland waters, from the infertile oligotrophic state to the more fertile eutrophic condition. Although man, by gross ill-treatment of the environment by uncontrolled additions of toxic pollutants, may upset this process, his damage may usually be reversed. But we must remember that eutrophic means well nourished, not polluted, and that eutrophic lakes contain much more life than those poor in nutrients. It is only that we prefer clean sterile water to rich pea soup.

The only point I would query in this book is its account of the plant succession in the development of woodland. We are told how abandoned farmland (originally made by clearing the forest) is invaded by weeds, then by shrubs and, perhaps only after many years, by the type of trees which comprised the original vegetation. But this is not always the case. In southern England the clay soils were mostly covered with oak forest before being cleared by Iron Age and Saxon farmers. When an area which has grown arable crops for hundreds of years is left alone, it is true that weeds like charlock immediately take over, to be replaced by shrubs (briar roses and hawthorn) in a few years. But oak seedlings, from acorns introduced by jays and other birds, may be present from the start, often occurring in very large numbers (many hundreds to the acre). Unless destroyed by the exotic rabbit, many of these survive and grow slowly and steadily, emerging from the herbaceous and shrubby vegetation as saplings in five to ten years. The succession is more apparent than real, for the main species of the climax vegetation may be present from the start.

However, this is a minor criticism, and probably does not apply to other forests with which I am unfamiliar. I consider that Professor Colinvaux has produced an excellent and very readable book. It should do much to widen the vision of professional ecologists and serious students, as well as educating the non-specialists for whom it was primarily written. □

Kenneth Mellanby is an Honorary Professorial Fellow in the Department of Botany at University College, Cardiff.

Characterisation of minerals

Physical Methods in Determinative Mineralogy. (Second edition.) Edited by J. Zussman. Pp. 720. (Academic: London, New York and San Francisco, 1977.) £23.50; $45.90.

There is no doubt that the first edition of this book, which appeared in 1967, met a widely-felt need for clear and concise descriptions of the most important techniques used in the separation, identification, and chemical and physical characterisation of minerals. The past decade has seen useful progress in the application of new methods of chemical analysis, and important advances in the

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physical (and particularly the structural) characterisation of minerals. To what extent does the second edition reflect these changes, and will it be as useful a servant to the mineralogists of the 1980s as the first edition was to those of the 1970s?

The list of contents of the second edition is little changed, two chapters (on automatic image analysis and neutron activation analysis) having been added, and one (on optical emission spectroscopy) deleted. The remaining chapters have been revised, sometimes by new authors. The extent of this revision may be judged by the increase in the proportion of new references, which ranges from about 20% for subjects such as mineral separation which have seen slow progress to 75% for those such as electron diffraction in which progress over the past 11 years has been very rapid.

A more detailed inspection of the revised chapters reveals particularly important changes in those dealing with reflected light miscroscopy, where quantitative reflectance measurements have increased in importance; with electron probe microanalysis, which has been affected by the introduction of energy-dispersive detectors; and with electron microscopy. There are useful improvements in most of the remaining chapters.

The new chapters, on image analysis and neutron activation analysis, will be of interest to many workers in both pure and applied mineralogy, and both techniques are likely to increase in importance during the currency of this edition. Both contributions are clear and well written.

With the increasing importance of physical methods of characterising minerals, a trend likely to continue during the life of this edition, it is a pity that more attention was not devoted to such subjects as modern single-crystal diffraction methods, including neutron diffraction. Despite this omission, the new edition undoubtedly represents a useful addition to the literature. The price is reasonable, but probably still high enough to deter individuals who own the first edition from buying the second. Libraries serving materials scientists concerned with minerals should not be so deterred, for there is much new material in the book.

R. G. J. Strens

R. G. J. Strens is a mineralogist working at the Institute of Lunar and Planetary Sciences, University of Newcastle upon Tyne, UK.

Plant molecular biology

Molecular Biology of Plant Cells. Edited by H. Smith. Pp. 496. (Blackwell Scientific: Oxford, 1978.) £13.50.

In the preface to this book the editor draws attention to the emerging enthusiasm and additional funding for basic research in plant biochemistry, cell physiology and genetics. This, he concludes, emphasises the need "for the training of competent research workers with specialist knowledge of plant cells, their structure, function, biochemistry and genetics". This book has been written with this kind of training in mind.

The seventeen chapters are divided into three major sections: "Plant Cell Structure and Function" (205 pages), "Gene Expression and its Regulation in Plant Cells" (178 pages), and the "Manipulation of Plant Cells" (48 pages). That some effort has been made to introduce a measure of uniformity into the way each of the chapters has been written is very evident. For example, in the first section each of the chapters has a brief introduction followed by descriptions of the structure of the particular cell components and their constitutent molecules followed by discussions on functions and mechanisms. Many but not all the chapters have a short summary.

Texts on plant biochemistry all too often fail to emphasise adequately the relationships between molecular structure and function. This is not one of them. Most, if not all, the authors of this book have paid particular attention to this important point.

Two chapters are devoted to membranes. One focuses on current models of membrane structure and how different kinds of molecules pass through them, but the second has, more unexpectedly, been included to emphasise the three-dimentional organisation and relationships of different membranes and the role endomembranes play in building the plant cell and integrating cellular activities. The inclusion of these chapters highlighting membrane functions is especially valuable, as current very interesting models of membranes as the primary action sites of auxin, gibberellic acid and phytochrome are introduced in other chapters devoted to the structures, functions and mechanisms of action of hormones and phytochrome.

To many molecular biologists, the lack of evidence in the text for the use of genetic variation in establishing relationships betwen structure, function and control mechanisms will be disturbing. This reflects the state of plant genetics, biochemistry and physiology rather than deficiencies in this particular book. The section on gene expression and its regulation opens with a brief sketch of the Jacob–Monod model for gene control in prokaryotes. This appearance of the details of prokaryotic gene control will probably serve to perpetuate erroneously the idea that the genes of higher plant are controlled in a similar way. Studies on fungi, which could have been more usefully highlighted, provide only little support for the relevance of the prokaryotic model for gene control in eukaryotes. Furthermore, there is ample evidence in the 178 pages of the second section on "Gene Expression and its Regulation" (covering the nucleus, protein synthesis, the regulation of enzyme levels and activity, hormone and phytochrome action) that hardly anything is known about gene control at the DNA level in higher plants.

As would be expected for a book on plant biochemistry, considerable space is devoted to chloroplasts. Two chapters ("Chloroplasts—Structure and Development", and "Chloroplasts—Structure and Photosynthesis") are included in the first section of the book, and a chapter on "The Genetic Information of Organelles and its Expression" is included in the second section. Together these chapters provide a good account of our knowledge of chloroplasts. Again their metabolic relationships to and dependence on other parts of the cell have not been neglected.

The final section of the book introduces that area of botanical research that surely has considerable promise for molecular biology—the culture of plant cells, protoplasts and the regeneration of whole plants from them. The exploitation of these systems in plant molecular biology has hardly begun. This is obvious from these chapters themselves and also from the paucity of information derived from cell cultures included in the other sections of the book.

I found the whole book very worthwhile reading. However, at £13.50, I suspect few undergraduates or research students will purchase it. This will perhaps be the biggest deterrent to it fulfilling its aims. Another serious difficulty with textbooks of this kind is their inevitable failure to be up to date. The emerging increased enthusiasm and additional funding for plant molecular biology has produced, in the past year or two, results which should be in a book of this kind. For example, the physical mapping of genes within the organelle genomes using restriction endonucleases is not mentioned, yet this approach and its natural developments will undoubtedly contribute considerably to plant molecular biology over the useful lifetime of this edition.

Richard B. Flavell

Richard B. Flavell is Principal Scientific Officer in the Cytogenetics Department of the Plant Breeding Institute, Cambridge, UK.

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Corticospinal control of movement

Corticospinal Neurones: Their Role in Movement. By C. G. Phillips and R. Porter. Pp. 450. (Academic: London and New York, 1977.) $32; £16.80.

THE cortical motor area was discovered by Fritsch and Hitzig in 1870. Its axons used to be thought of as providing a straightforward passageway, the pyramidal tract, to the spinal segments, but one without exclusive right to engage the executives of Sherrington's final common pathway, today's alpha motoneurones. The Nauta stain in the hands of Kuypers (Rotterdam) has revealed a surprising wealth of emissions from the axons along this tract (as shown in his excellent diagram). For the cells in the cortical motor area whose axons branch off in the internal capsule, the cerebral peduncles and the pons the authors of the book reviewed here introduce the term "parapyramidal"; Starlinger's old term "extrapyramidal" is reserved for neurones located outside the area of origin of the pyramidal tract. The main aim of this book is to analyse and summarise what is known about the anatomy and physiology of the corticospinal neurones and the pyramidal tract.

An attractive feature of the work is that the authors repeatedly return to a discussion of the history of their subject matter before proceeding to descriptions of experiments using microelectrodes on single cells at rest and in activity, of microanatomy, and so on. Room has been found for every contribution of interest and most leading experiments have been closely scrutinised to sort out hard evidence from hypotheses and notions.

Both authors have made decisive contributions to cortical motor physiology. Phillips was in fact pioneering cortical microphysiology in the mid-1950s, making intracellular recordings from the large Betz cells of the cortical motor area. His major achievement (working with a large team of coworkers) has been the function and has provided into the function and organisation of corticospinal control in primates, which he has built up from studies of identified pyramidal tract neurones acting on motoneurones impaled for intracellular analysis. One of his coworkers was Porter, who, after returning to Monash University, returned to experiments on cortical cells in freely moving monkeys, adding new aspects to the pioneer studies of Evarts at the National Institutes of Health in Bethesda, Maryland, and of Brooks in London, Ontario, on monkeys trained to execute arm and hand movements against controlled forces.

The authors state that they have attempted to follow four main avenues for discussion: effects of electrical stimulation of the brain; the mapping of the cortical neural connexions, including a full survey of all the methods used, anatomical as well as physiological; electrical recording of neural activity during specific performances; and description and analysis of normal performance and of the effects of central and peripheral disturbances upon it. They have managed to achieve this and a great deal more. Their presentation is stuffed with well-digested information; here, I can only try to indicate what kind of organisational and functional role for the corticospinal neurones in movement emerges from their analysis of classical and contemporary experimentation.

By far the most important contributions have come from electrophysiological methods of stimulation and intracellular recording. These receive the lion's share of the authors' attention. The cortical motor area has always been held to represent a map of the body musculature. On this area, the muscles are projected in an orderly manner, and those which perform discrete, delicate movements (like the hand and finger muscles) occupy far more space (and therefore more cortical neurones) than others of less importance in this respect. The combination of muscle actions in numerous ways during activity is made possible by the fractionation of the motor area into overlapping groups or "colonies" of cortical cells projecting to the same spinal motoneurones. The projections of corticospinal axons form efficient synapses, capable of high frequency response, on spinal motoneurones. The same cells also receive a monosynaptic input of slightly different character from the muscle spindles. When in voluntary movement the gamma motoneurones for the spindles are coactivated with the alpha motoneurones, the latter are boosted through the gamma loop. Together the two routes of activation form a control system available for rapid specific action during delicate movements such as the precision grip of the primates. Lower mammals do not possess the monosynaptic variety of corticospinal contact with motoneurones, and therefore do not have the precision grip.

The small neurones of the motor area are spontaneously active but their activity is modulated less during body movement than that of the larger ones, whose axons have high conduction velocity. These latter fire repetitively to determine the extent and rate of change in the forces involved in body movement; their activity can also be modified by external stimuli in the form of instructions to the animal, even when the latter take effect by a delayed afferent trigger signal. Forces involved in voluntary body movement elicit stretch reflexes, the secondary component of which have a latency compatible with conduction through a transcortical loop.

This book will be for some time to come an indispensable companion for all those seriously interested in how the brain works and in the limits of our present knowledge in the field of motor control.

Ragnar Granit

Ragnar Granit is at the Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm.

Physics of liquids

Progress in Liquid Physics. Edited by C. A. Croxton. Pp. 592. (Wiley: London, New York and Toronto, 1978.) £25.

IT is quite appropriate to say that in this field there has been remarkable "progress" in the past few years, especially in the *theory* of liquids, with which this book is mostly concerned. Several other books on liquids have been published recently which give a general introduction to the subject (Hansen and McDonald, Watts and McGee, March and Tosi, Croxton). But this one is different in that it is a collection of high level reviews of various particular topics.

The volume is not the first of a series, apparently, although there are enough important topics missing to fill at least one more volume, as the field of liquids is so rich in the experimental and theoretical techniques in common use. The authors are all outstanding experts in their particular field and the accounts are authoritative and well documented with numerous references. This is not a browsing book or an introductory one and will be most used to obtain a view of a particular topic as the need arises.

The book was completed in about January 1977 and the reader should note that in many topics some important advances have been made since then. For once, however, the price does not seem too excessive for 592 pages of well-printed text, especially as the book is likely to be purchased mainly by libraries as a reference work.

J. G. Powles

J. G. Powles is Professor of Physics at the University of Kent, Canterbury, UK.

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Chromatographic fractionation of polymers

Chromatography of Synthetic and Biological Polymers. Vol. 1: Column Packings, GPC, GF and Gradient Elution. Edited by Roger Epton. Pp. 386. (Wiley: London and New York, 1978.) £18.

THIS book is the first of a two-volume set devoted to the chromatographic fractionation of naturally occurring and synthetic polymers. The first volume is confined wholly to separations depending on differences in molecular dimensions, methods which are referred to in synthetic polymer science as gel permeation chromatography (GPC) and in biochemistry as gel filtration (GF). Neither term is logical as both methods may use rigid stationary phases such as porous glass or silica, and the connotation of "permeation" and "filtration" is at best vague. I prefer the directly descriptive terms, molecular sieve chromatography or more briefly, exclusion chromatography, which are equally applicable to both groups of separations.

The fields of application to synthetic and biological polymers have become so diversified that the decision to include both in a single publication is itself questionable. This diversity arises, not as the editor suggests in his introduction, from the differences in the scientific backgrounds of the investigators, but rather from the intrinsic differences in the chemistry of synthetic and biological polymers. The former are usually weakly polar or non-polar and mostly water-insoluble, whereas the latter are polar, frequently ionised, and generally soluble only in aqueous media. This fundamental difference has necessitated corresponding differences in the stationary phases used for separations in the two fields, so that very few are applicable to both. In this book (the proceedings of a conference held in Birmingham in 1976), 16 of the 28 individual communications deal exclusively with synthetic polymers, ten with biological polymers, and two with both fields.

Part 1 (papers 1-11) is the most valuable section of the book, as it provides a comprehensive account of the column packings used for exclusion chromatography, many of them novel and reviewed here in detail for the first time. These include the Ultrogels, composite agarose–polyacrylamide gels, the Enzacryls, poly(acryloyl morpholine) gels, and the Spherons, poly (hydroxyethyl methacrylate) gels. The latter two media are compatible with both aqueous and organic solvents, whereas the Spherons (like the bead-form celluloses described in chapter 6) are readily converted into ion-exchange derivatives.

Part 2, on preparative and industrial scale chromatography, deals with scaling-up methods (chapter 12), and the intriguing topic of continuous flow chromatography is well reviewed in chapter 13 by Barker and his associates, who have pioneered in this field. I found chapter 14 disappointing, as despite its title, it deals only briefly with gradient elution as generally understood in separation science, and predominantly with synthetic polymer fractionation in a temperature gradient field.

Part 3, on applications, provides the usual miscellany of papers presented at conferences of this type. A notable exception is chapter 18 by Brewer and Soderberg on fractionation of proteins between a cross-linked dextran stationary phase and a mobile phase containing water-soluble polymers. This short paper is of outstanding importance, as it promises to provide a means of applying the powerful Albertsson polymer two-phase liquid partition systems to chromatography. The Albertsson systems are unequalled in selectivity and range of application.

In summary this volume, despite its specialised appeal to two dissimilar groups of readers, provides the best account of recent advances in exclusion chromatography now available, and is warmly recommended.

C. J. O. R. Morris

C. J. O. R. Morris is Emeritus Professor of Experimental Biochemistry, formerly at the London Hospital Medical College and Queen Mary College, University of London, UK.

Collision spectroscopy

Collision Spectroscopy. Edited by R.G. Cooks. Pp.458. (Plenum: New York, 1978.) $54.60.

PROCESSES like collisions between ions and molecules at high energies (in the kilovolt range), play a predominant role in the charge balance in the ionosphere as well as in laboratory plasmas. Collisional activation has recently been applied to metastable ions in mass spectroscopy to derive from them energy-differentiated spectra which can be used for the identification of fragment ions leading to the elucidation of the structures of complex organic molecules and the mechanisms of gaseous ion reactions. This book is intended to review a variety of studies concerned with these topics.

Following an introductory chapter by the editor there are seven chapters written by specialists on different aspects of the subject. The first covers electronic excitation of the ion or the target molecule in elastic collisions. It describes the two major experimental techniques for these studies: the energy-loss spectra of the primary particle itself; and the detection of photons emitted from the excited states resulting from the collision. Chapter 2 covers charge transfer in atomic systems from both a theoretical and experimental point of view. Information on potential energy functions for these transfer reactions is obtained by measuring cross sections which are differential in both scattering angle and kinetic energy.

The third chapter, dealing with both charge exchange and excitation reactions, emphasises the value of translational energy measurements as a source of data on the primary collision event. It discusses a molecular orbital theory of inelastic collisions which seeks to construct time variable molecular orbitals for the dynamic system. It also describes the time-of-flight techniques which have been used to study the fast neutral products of inelastic collisions and has an important section on ion–photon and photon–photon coincidence techniques. Chapter four deals with the charge inversion of positively charged ions, the reaction being characterised in terms of the kinetic energy spectra of the product anions. Chapter five shows how several modified types of mass spectra which depend on the occurrence of ion–molecule interactions can be recorded. By appropriate adjustment of the electrostatic analyser of a mass spectrometer it can readily be arranged that only ions of a certain energy to charge ratio will be transmitted. The resulting spectra are a unique source of information on the structure and chemistry of polyatomic ions. Chapters 6 and 7 continue the study of collision-induced dissociation in diatomic and polyatomic ions and its application in mass spectrometry.

The book covers a rapidly growing and important subject which has many different facets. It performs the valuable service of integrating these in an authoritative way.

W. C. Price

W. C. Price is Emeritus Professor of Physics at King's College, University of London, UK.

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obituary

J. B. Conant, 1893-1978

Harvard University Archives

JAMES BRYANT CONANT, notable American chemist, innovative president of Harvard University, organiser and principal leader of the American wartime technological efforts, postwar science statesman, ambassador to Germany and at the age of seventy the most influential but controversial school reformer, died at the age of eighty-four on 11 February, 1978 after a long illness. He leaves behind his wife, née Grace Thayer Richards, whom he married in 1921, two sons and five grandchildren.

Jim Conant, the son of a photoengraver, was born in Dorchester (now a part of Boston) on 26 March 1893 was educated in local public schools and entered Harvard college in 1910. He planned his undergraduate curriculum for a career in chemistry, completed the four-year course with distinction in three and yet found time to be the editor of the *Harvard Crimson*, the undergraduate daily paper.

His graduate work was also at Harvard and led to a Ph.D. degree in 1916. It combined a project in physical chemistry under Professor T. W. Richards, the Nobel laureate known for his atomic weight determinations and a synthetic organic problem under Profesor E. P. Kohler. Then followed war work, involving research on the synthesis of Lewisite, an arsenical poison gas, and the construction of a plant to manufacture it.

Conant returned to Harvard in 1919 as an instructor in organic chemistry. Organic chemistry then was totally dominated by European scientists: Richard Willstätter, Leopold Ruzicka, Emil Fischer, Robert Robinson, Arthur Lapworth, and many others. Conant, almost alone among American chemists, came to the forefront of both major thrusts of organic chemistry, natural products and fundamental theory. Although he himself regarded his research on the structure of chlorophyll as most significant, his contributions to physical-organic chemistry had a much greater impact on subsequent developments, since Conant's ideas and discoveries stimulated rapid progress in the understanding of reaction mechanisms.

In particular, he and N. F. Hall introduced the concept of superacidity for solutions of strong acids in non-aqueous solvents. With G. M. Gramann Conant showed that both sodium acetate and perchloric acid strongly catalyse the acetylation of β-naphthol in acetic acid. Sodium acetate had, of course, already been used by synthetic chemists as a catalyst, but the understanding that it functions as a strong base in acetic acid as solvent was of fundamental importance to future work. Conant and G. W. Wheland initiated the quantitative treatment of extremely weak acids such as cyclopentadiene, using triphenylmethyl sodium in ether as the needed strong base. These were germinal contributions to the theory of non-aqueous solutions.

Conant's investigations with L. F. Fieser, among others, of the reversible oxidation-reduction potentials of quinones, and his studies of irreversible electrochemical oxidations and reductions were far ahead of their time. In his work with P. D. Bartlett on the mechanism of semicarbazone formation, he distinguished clearly between kinetic and thermodynamic controls and that paper exerted a powerful influence on developing theory. He and Professor P. W. Bridgman were the first to investigate the effects of extremely high pressures on the rates of organic reactions, discovering a striking acceleration of polymerisations.

Among Conant's contributions in the biochemical area was the discovery of copper in the prosthetic group of hemocyanin, the oxygen-carrying pigment of the blood of crustaceans. In his major biochemical work, Conant and his collaborators discovered the role of autoxidation in the so-called 'phase test' for chlorophyll—a complex and previously confusing series of reactions initiated by strong bases. Although Conant's part in the chlorophyll story was not the central one, Hans Fischer having proposed in 1935 a substantially correct structure, one can only speculate as to what Conant might have accomplished had he not been elected president of Harvard in 1933. Also in that year appeared the first edition of his influential textbook, *The Chemistry of Organic Compounds*; he had already been the editor-in-chief of the second volume of the internationally acclaimed series *Organic Syntheses*.

As president of Harvard University Conant still found time to maintain an active interest in chemistry. Thus he participated in the interpretation of the heats of organic reactions being measured by a group led by G. B. Kistiakowsky and he stimulated a group led by A. B. Hastings and

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G. B. Kistiakowsky to apply the short-lived ^{11}C, the only radioactive isotope of carbon then available, to a study in depth of a metabolic pathway in the liver.

During the thirteen years that Conant was an academic chemist more than sixty young men and women published research papers with him. Several among his students became distinguished academic scientists: P. D. Bartlett, L. F. Fieser, A. M. Pappenheimer, Frank Westheimer at Harvard, G. W. Wheland at the University of Chicago, J. G. Astin at the Pennsylvania State University, A. Corwin at Johns Hopkins University, and others.

Entering the presidency, Conant found Harvard little more than a New England college; he left it twenty years later the leading national university. Throughout life Conant was a scholar dedicated to research and the measures he introduced at Harvard emphasised research and graduate education as comparable in importance with undergraduate instruction. A major role in this transformation was the limitation of all non-tenure faculty appointees to a maximum of eight years and the introduction of *ad hoc* committees composed mainly of scholars from other institutions to screen all candidates for tenure appointments at Harvard on the basis of their scholarly contributions. Many American universities subsequently adopted similar rules and procedures.

Conant created the University Professorships to free a few distinguished faculty scholars from routine duties. He thoroughly reorganised the Harvard engineering school into a Division of Applied Physics and Engineering Sciences. Through his selection and encouragement of Francis Keppel as the Dean of a limping Graduate School of Education the latter was transformed into the leading centre for the training of school administrators.

Among the many reforms introduced into the undergraduate education the most important were those proposed in the 1945 faculty report *General Education in a Free Society*. This report, known as the 'Red Book', exercised great influence on educational programmes in colleges throughout America. Conant also created National Scholarships at Harvard for needy students from all parts of the United States to broaden the student body from one of predominantly New England origin. After the war he eliminated separate lectures and laboratory instruction for the Harvard and (female) Radcliffe undergraduates.

In the intercollegiate ('varsity') sports, which play a large role in American colleges, Conant replaced an autonomous body led by the alumni association, which is the norm in American universities and is the source of athletic scholarships, by a departmental structure administered by a faculty committee. The scholarships to athletes *qua* athletes were eliminated.

In the late thirties Conant was the first academic leader in America to join a public debate and urge military preparedness in the face of threats posed by Hitlerism. In the fall of 1939 he was already discussing ways by which American scientists could assist the British war effort, thus also preparing themselves for the time when their technical services would be needed at home. Next spring he joined Vannevar Bush in organising the National Defense Research Committee (NDRC), a civilian organisation felt to be necessary because the American military research establishment was wholly fossilised by neglect and lack of funds.

Conant became the head of the chemical division of NDRC, but, as he told me then, he knew that 'World War I was the war of the chemists, this one will be the physicists' war' and so he advised me to concern myself with explosives and ordnance, not chemical warfare.

The politics of American isolationism and the dangers of trans-Atlantic travel in 1940 engendered strong opposition to Conant's proposal to establish a systematic exchange of technical information and personnel between NDRC and the British military-technical establishment. Conant overcame the opposition and in February 1941 led a mission to England, where he was hailed as a messenger of hope by the beleaguered nation. The task of establishing effective channels of information exchange was quite complex because the United States was still expressly neutral, because NDRC was a young organisation composed largely of non-government civilians and because at that time the information had to flow mostly one way, westward, the British military technology being well-ahead. Conant succeeded in overcoming these obstacles beyond his own fondest hopes. In the process he was received by the King, had two meetings and meals with the Prime Minister and established cordial relations with the leaders of the British military-technical establishment.

Departing England a month later, having had an automobile accident while being driven to Chequers by Professor R. G. W. Norrish, also 'flu and a severe head cold induced by overwork and under-heated rooms, he left behind a working London NDRC liaison office. This group became highly effective in ferreting out information and in smoothing the way for visiting American scientists and engineers who followed Conant as a trickle in the spring of 1941 that grew to a flood in later years.

In the meantime the NDRC organisation grew fast; President Roosevelt, to emphasise its importance, created a parent body, the Office of Scientific Research and Development, a part of the White House staff. Bush became its director, Conant his deputy and his successor as the chairman of NDRC. While Bush was mainly involved in the high councils of government and managing the relations between NDRC and the military. Conant became the head organiser and a hard-driving manager of a great variety of war-related NDRC projects numbering into the thousands.

The largest group efforts went probably into microwave radar, sonar, electronic countermeasures, proximity fuses, barrage rockets, aids to amphibious operations, a new process for manufacturing and using the British RDX explosive and, of course, uranium fission, which, however, soon ceased to be a part of NDRC. To mid-1943, Conant was the chairman of a special committee administering the uranium project and he took personal responsibility for the big gamble of nearly direct transition from laboratory scale work to the giant industrial establishments for the production of fissionable materials. When General L. E. Groves took over for the Army Corps of Engineers in 1943, a top level Military Policy Committee was established by Roosevelt, chaired by Bush with Conant his alternate, to which General Groves was to report. It fell to Conant to monitor the progress of the complex parts of what became known as the Manhattan District and to resolve conflicts between 'the most colossal egos' with whom he ever had to deal, as he once confided to me. To Groves Conant appeared to have the courage of his Yankee forebears.

At war's end Conant returned to Harvard expecting to be again its full-time president. Washington, however, had other plans for him. He was persuaded to accompany Secretary of State Byrnes to Moscow to negotiate agreement on international control of atomic energy which Conant advocated strongly. To the visitors' surprise the Soviet government accepted the American proposals. Had not the other Soviet-American confrontations induced President Truman to withdraw his support of Byrnes' initiative, the future course of Soviet-American relations might have been quite different.

Conant also became active in the debate on the structure of the American atomic energy establishment, supporting the proposals of the May-

Johnson bill, then before Congress. This earned him the enmity of the liberal wing of atomic scientists who regarded this bill as militaristic. The May–Johnson bill was vetoed by President Truman and later the Atomic Energy Commission of the McMahon bill came into being. Conant was appointed a charter member of its General Advisory Committee which, for several years to follow, was a very influential group chaired by Oppenheimer. He joined the latter in opposing crash development of the 'super', the hydrogen bomb. When Oppenheimer was threatened with the loss of his military security clearances, being accused of indiscretions such as friendship with some members of the American Communist party, by the military and civilian proponents of the *H*-bomb, led by Edward Teller, Conant took a strong stand and testified on Oppenheimer's behalf at the scandalous hearings before an AEC security board that led to Oppenheimer's dismissal.

Conant became convinced during the war that to insure social progress the Federal Government in peacetime must finance basic scientific research in academic institutions and not do it through its military agencies, because of their secrecy requirements and emphasis on short range goals. At Harvard he established the rule that all faculty research must be open to publication thus closing the doors to most military research contracts. In Washington he took an active part in Congressional hearings which eventually led to the establishment of the National Science Foundation in 1949. Conant became the chairman of its policy-making body, the National Science Board. There he played an important role in the development of NSF policies and procedures in support of basic research which have largely survived to this day.

Conant was offered and accepted the nomination to the presidency of the National Academy of Sciences. However, the liberal elements among atomic scientists disapproved of his stand on the bombing of Japan and his position on the structure of the atomic energy agency. Other elements of the scientific community, including those who were attacking Oppenheimer, were resentful of Conant because of decisions he took in the course of his firm leadership of NDRC and the Manhattan project. Discovering the coalescence of these enmities into open opposition Conant withdrew his candidacy, to a grave disappointment of his many friends. Two years later he retired from the presidency of Harvard, to be appointed United States High Commissioner for Germany and this signaled the end of all his ties with the scientific community.

For four years, as the High Commissioner and then the Ambassador in Bonn, Conant worked effectively with Konrad Adenauer to transform defeated Germany into the Federal Republic, a cornerstone of the NATO alliance.

Sensing this task accomplished Conant returned to the United States in 1957 to embark on the last major career of his extraordinary life. While as president of Harvard he wrote and spoke frequently on problems of college education, Conant turned now to American high schools, assisted by a grant from the Carnegie Foundation. That happened at the time of the Sputnik when doubts about the adequacy of American education were rampant in the United States.

Two years later his book *The American High School* came out and soon more than 250,000 copies of it were in circulation. The book contained explicit recommendations for improving education and Conant embarked on nationwide travels strenuously campaigning for these reforms.

In 1961 Conant published an angry book *Slums and Suburbs,* in which, well ahead of the widespread unrest of the late sixties, he warned that the growing numbers of school dropouts and other unemployed urban youths in congested slums constituted a growing charge of social dynamite. This book caused Conant to be attacked by many black leaders because he advocated jobs ahead of school integration. As Conant notes in his autobiography his mistake was not to seek 'alliance with those black leaders who found my book objectionable.'

Two years later came the publication of his last book of this period, *The Education of American Teachers,* dealing with what he regarded as their scandalously low educational standards. As Conant anticipated the entire school teachers' establishment rose in arms to attack him furiously, but some reforms did follow later.

Prior to final retirement Conant spent two years in West Berlin as the advisor to that city's government, on an educational project financed by the Ford Foundation, and then returned to the United States to have a second look at public schools and publish his findings. Altogether he wrote ten books dealing with public education in addition to those on chemistry. He died in Hannover, New Hampshire, his summer home.

What remains now is the astonishing record of a man who lived many lives and achieved much in each. Conant was one of the principal architects of the American scientific edifice. He combined inborn conservatism with great intellectual boldness and the courage of his convictions when dealing with educational problems. As a high level manager of R & D he had an unerring feeling for what was right and wrong. He was a cool Yankee and yet could be a warm friend. The presence of Jim Conant made an indelible mark on this century's America.

G. B. Kistiakowsky

G. J. Bramwell

GEOFFREY JOHN BRAMWELL, Lecturer in Pharmacology in the Department of Pharmacy, Nottingham University, died of leukaemia on 13 April 1978, at the age of 31. Born at Leamington Spa, he was educated at Warwick School and the School of Pharmacy, London, where he graduated in 1969 with a B.Pharm.(Hons) and then went on to work for a Ph.D. (University of London) which he received in 1973. He spent two years as a Research Fellow in the Department of Pharmacology, University of Birmingham, before moving to Nottingham in 1974.

Geoff Bramwell's research work centred on the pharmacology of neurones in the central nervous system and he was interested in the interactions between neurotransmitter substances and drugs with central effects, utilising the technique of microiontophoresis. His early work in Donald Straughan's department at the School of Pharmacy, was to investigate the actions of drugs on 5-hydroxytryptamine-containing neurones in the brain, particularly cells in the raphe nuclei. The drugs he studied included LSD and, although this work resulted in only a small number of publications, he made a significant contribution in this area. On moving to Birmingham, Bramwell became involved in studies on the actions of opiate drugs on single neurones in the brain and he continued this work at Nottingham after first setting up a laboratory for electrophysiological studies. He extended his studies to include the opioid peptides and latterly became interested in the quantitative aspects of neuronal responses to locally applied pharmacological agents. He published in the *British Journal of Pharmacology, Brain Research* and *Neuropharmacology,* and his work was becoming known internationally at the time of his death.

Very few of his friends and colleagues knew of his illness and, in spite of the difficulties caused by the therapy, he remained cheerful and was strenuously trying to keep his research work going. He is survived by his wife, Alison.

P. B. Bradley

announcements

Meetings

2–7 July, **4th International Meeting on NMR Spectroscopy,** York (Dr J. Gibson, The Chemical Society, Burlington House, London W1, UK).

3–7 July, **An Introductory Course in Tropical Hygiene,** London (Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel St (Gower St) London WC1, UK).

3–13 July, **Symposium on Mathematical Theory of Non-Linear problems in Quantum Mechanics and Quantum Optics,** Durham (E. A. Power, Mathematics Dept, University College, London, Gower St, London WC1, UK).

12–14 July, **AGM of the Biochemical Society,** London (The Biochemical Society, 7 Warwick Court, High Holborn, London WC1, UK).

15–20 July, **7th International Congress on Thrombosis and Haemostastis,** London (Phase 2 Contracting Services Ltd, Congress Exhibition Dept, Rusham Rd, Egham, Surrey, UK).

15 July, **A multifactorial Approach to Causes and Treatment of Learning Disabilities in Children,** London (ACLD, 27 Hampton Rd, Folly Hill, Farnham, Surrey, UK).

17–21 July, **International Nutrition: Problems, Policies and Strategies,** Massachusetts (Jane Hogan, INP, Massachusetts Institute of Technology, 20A–222 18 Vassar St, Cambridge, Massachusetts 02139).

18–20 July, **Conference on Artificial Intelligence,** Hamburg (Derek Sleeman, Dept of Computer Studies, The University, Leeds 2, UK).

20–21 July, **Hormones and Food Utilisation,** Southampton (Dr M. R. Turner, British Nutrition Foundation, 15 Belgrave Square, London SW1, UK).

23–25 July, **Pharmacological Modulation of Steroid Action,** Turin (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23 Milan, Italy).

23–29 July, **Photosynthesis and Plant Development,** Diepenbeek (Dr R. Marcelle, Laboratory of Plant Physiology, Research Station of Gorsem, B 3800 SINT TRUIDEN? Belgium).

24–26 July, **Coordination Chemistry and Cancer Chemotherapy,** Toulouse (Prof. F. Gallais, Laboratoire de Chimie de Coordination et de Pharmacologieet Toxicologie fondamentales du CNRS, Toulouse, France).

24–28 July, **4th International Congress of Pesticide Chemistry,** Zurich (Congress Secretariat, 4th International Congress of Pesticide Chemistry, PO Box 182, CH-4013 Basle, Switzerland).

Reports and Publications

Other countries—April

United States Department of the Interior: Geological Survey. Water-Supply Paper 2164: Ground-Water Levels in the United States, 1973-74. Northeastern States. Pp.v + 126. (Washington, DC: US Government Printing Office, 1977.) [134

United States Department of the Interior: Geological Survey. Bulletin 1430: Mineral Resources if the Proposed Additions to the Scapegoat Wilderness, Powell and Lewis and Clark Counties, Montana. By Robert L. Earhart, David J. Brimes, Reinhard W. Leinz and Lawrence Y. Marks. Pp. vii + 62 + 2 plates. (Washington, DC: US Government Printing Office, 1977.) [174

Energy, Mines and Resources Canada. Geological Survey of Canada. Bulletin 273: Multiple Glaciation in the Area of Williston Lake, British Columbia. By N. W. Rutter. Pp. 31. (Ottawa: Geological Survey of Canada, 1977.) $6. [174

United States Department of the Interior: Geological Survey. Bulletin 1397-C: Mineral Resources of the Ramseys Draft Wilderness Study Area, Augusta County, Virginia. By Frank G Lesure, Philip J. Geraci, Peter C. Mory and Bradford B. Williams. Pp. vi + 42. Professional Paper 997: Paleogene Floras From the Gulf of Alaska Region. By Jack A. Wolfe. Pp.iv + 108 + 30 plates. Professional Paper 1040: Stochastic Analysis of Particle Movement over a Dune Bed. By Baum K. Lee and Harvey E. Jobson. Pp. vi + 72. (Washington, DC: US Government Printing Office, 1977.) [184

Worldwatch Institute. Worldwatch Paper No. 19: The Solar Energy Timetable. By Denis Hayes. Pp. 40. (Washington, DC: Worldwatch Institute, 1776, Massachusetts Avenue, N.W., 1978.) $2. [184

Republic of South Africa. Department of Agricultural Technical Services. Technical Communication No. 136: Modern Trends in Methods of Food Production, Food Processing and Food Preparation which Constitute a Potential Hazard to Human and Animal Health. By D. G. Steyn. Pp. 48. (Pretoria: Department of Agricultural Technical Services, 1977. Obtainable from the Director, Division of Agricultural Information, Private Bag X144, Pretoria.) [184

Fiskeridirektoratets Skrifter. Serie Havundersøkelser, Vol. 16, No. 10: Stock Size Fluctuations and Rate of Exploitation of the Norwegian Spring Spawning Herring, 1950-1974. By Olav Dragesund and O. Ulltang. Pp. 315-337. (Bergen, Norway: Directorate of Fisheries, 1978.) [194

Smithsonian Contributions to Paleobiology, No. 34: Index of Living and Fossil Echinoids, 1924-1970. By Porter M. Kier and Mary Hurd Lawson. Pp. vi + 182. (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office.) [194

Lawrence Berkeley Laboratory 1978. Pp. 89. (Berkeley, CA: Lawrence Berkeley Laboratory, University of California, 1978.) [194

Clinical and Experimental Hypertension, Vol. 1, No. 1, 1978. Edited by Dr. Michael J. Antonaccio. Pp. 1-140. Subscription rate for Volume 1, 1978, containing 6 issues $90 (prepaid). Add $8.10 per volume for postage outside the United States. (New York: Marcel Dekker, Inc., 270 Madison Avenue, 1978.) [244

The Human Genetic Mutant Cell Repository: List of Genetic Variants, Chromosomal Aberrations and Normal Cell Cultures Submitted to the Repository. Fourth edition. Pp. ix + 171. (Institute for Medical Research, Camden, NJ.) (Bethesda, Md.: U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1977.) [244

United States Department of the Interior: Geological Survey. Water-Supply Paper 2161: Ground-water Levels in the United States, 1971-74. Northwestern States. Pp. v + 153. Bulletin 1435-C: The Pliocene Conant Creek Tuff in the Northern Part of the Teton Range and Jackson Hole, Wyoming. By Robert L. Christiansen and J. D. Love. Pp. iii + 9. (Washington, DC: US Government Printing Office, 1977 and 1978.) [244

Smithsonian Contributions to Zoology. No. 250: West African Myodocopid Ostracoda (Sarsiellidae, Rutidermatidae). By Louis S. Kornicker and Francisca Elena Caraion. Pp. 110. (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office.) [254

World Meteorological Organization. Technical Notes. No. 150: Application of Building Climatology to the Problems of Housing and Building for Human Settlements. By J. K. Page. Pp. xvi + 64. No. 151: Crop-Weather Models and Their Use in Yield Assessments. By Wolfgang Baier. Pp. xviii + 48. No. 152: Radiation Regime of Inclined Surfaces. By K. Ya. Kondratyev. Pp. xiv + 82. No. 153: The Use of Satellite Imagery in Tropical Cyclone Analysis. Pp. 84. No. 154: The Scientific Planning and Organization of Precipitation Enhancement Experiments, with Particular Attention to Agricultural Needs. By J. Maybank. Pp. xvi + 88. No. 155: Forecasting Techniques of Clear-Air Turbulence Including That Associated with Mountain Waves. By Robert H. Hopkins. Pp. 31. No. 156: Effects of Human Activities on Global Climate. By William W. Kellogg. Pp. cviii + 47. (Geneva: World Meteorological Organization, 1977.) [254

Bellagio IV Population Conference. (A Conference Sponsored by The Rockefeller Foundation. June 7-9, 1977, Ulvshale, Denmark.) Pp. ix + 169. (Working Papers.) (New York: The Rockefeller Foundation, 1133 Avenue of the Americas, 1977.) *gratis*. [264

Institutt for Atomenergi, Kjeller. Kjeller Report-155: A Data Recording Processing System for Examination of Irradiated Fuel Rods—Description, User's Guide and Examples. By K. D. Olshausen, J. F. Christiansen, J. K. Larsen and T. Stevens. Pp. 70. (Kjeller, Norway: Institutt for Atomenergi, 1978.) [264

Taxonomy in Europe. (Interim Report of a Study by an ESRC ad hoc Group. ESRC Review No. 13. Committee of European Science Research Councils.) Pp. 95. (Strasbourg, France: European Science Foundation, 1978.) [274

World Health Organization. International Classification of Procedures in Medicine, Vol. 1. (Published for trial purposes in accordance with resultion WHA 29.35 of the Twenty-ninth World Health Assembly, May 1976.) Various paginations. (Geneva: World Health Organization; London: HMSO, 1978.) Sw.fr. 15; US$6.75. [284

United States Department of the Interior: Geological Survey. Water-Supply Paper 2165: Ground-Water Levels in the United States, 1974. Southeastern States. Pp.v + 116. Professional Paper 1019: Climatography of the Front Range Urban Corridor and Vicinity, Colorado; a Graphical Summary of Climatic Conditions in a Region of Varied Physiography and Rapid Urbanization. By Wallace R. Hansen, John Chronic and John Matelock. Pp. iv + 59. (Washington, DC: US Government Printing Office, 1977 and 1978.) [284

UK & Ireland—May

The Council of Engineering Institutions. Annual Report for 1977. Pp. 40. (London: The Council of Engineering Institutions, 2 Little Smith Street, SW1, 1978.) [25

The Future of the Public Analysts Scientific Advisory and Analytical Services. Prepared by the Association of Public Analysts. Pp. 8. (Supplement to Volume 16 of the *Journal of the Association of Public Analysts*.) (London and New York: Academic Press, 1978.) [25

National Radiological Protection Board. Harwell. NRPB-R69: Preliminary Assessment of the Radiological Protection Aspects of Disposal of High-Level Waste in Geological Formations. By M. D. Hill and P. D. Grimwood. Pp. iii + 75. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1978. Obtainable from HMSO, London.) £2. [25

Water Analysis: Some Revised Methods for Limnologists. By F. J. H. Mackereth, J. Heron and J. F. Talling. (Scientific Publication No. 36.) Pp. 120. (Ambleside, Cumbria: Freshwater Biological Association, The Ferry House, Far Sawrey, 1978.) £2.50. [45

Report of the Centre for Overseas Pest Research, January–December 1976. Pp. vi + 150. (London: Centre for Overseas Pest Research, College House, Wrights Lane, W8, 1978.) £3. [45

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